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Henri Matisse (1869-1954).
Icarus
(from the illustrated book, Jazz, published in 1947 by E. Tériade).

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Planning against Biological Terrorism: Lessons from Outbreak Investigations

David A. Ashford,* Robyn M. Kaiser,* Michael E. Bales,* Kathleen Shutt,* Amee Patrawalla,* Andre McShan,* Jordan W. Tappero,* Bradley A. Perkins,* and Andrew L. Dannenberg*

We examined outbreak investigations conducted around the world from 1988 to 1999 by the Centers for Disease Control and Prevention's Epidemic Intelligence Service. In 44 (4.0%) of 1,099 investigations, identified causative agents had bioterrorism potential. In six investigations, intentional use of infectious agents was considered. Healthcare providers reported 270 (24.6%) outbreaks and infection control practitioners reported 129 (11.7%); together they reported 399 (36.3%) of the outbreaks. Health departments reported 335 (30.5%) outbreaks. For six outbreaks in which bioterrorism or intentional contamination was possible, reporting was delayed for up to 26 days. We confirmed that the most critical component for bioterrorism outbreak detection and reporting is the frontline healthcare professional and the local health departments. Bioterrorism preparedness should emphasize education and support of this frontline as well as methods to shorten the time between outbreak and reporting.

Bioterrorism is the intentional use of microorganisms or toxins derived from living organisms to cause death or disease in humans, animals, or plants on which we depend. In 2001, *Bacillus anthracis* was disseminated through the U.S. postal system (1). Before that event, concern about bioterrorism had led to preparedness efforts, including strategic planning (2). As part of these efforts, we examined investigations conducted by the Centers for Disease Control and Prevention's (CDC) Epidemic Intelligence Service (EIS). EIS was established after World War II, in part to protect the United States against bioterrorism. We reviewed characteristics and trends of EIS investigations conducted from 1988 to 1999 (3). Outbreak investigations from 1946 to 1987 had already been reviewed (4). We focused on field investigations involving agents that could potentially be used for bioterrorism because understanding how these outbreaks were detected and reported might improve early detection and reporting of bioterrorism.

Each EIS field investigation follows an official request from a state or international health agency. States and international health agencies receive reports of cases or outbreaks from many sources, including local public health agencies, hospitals, healthcare providers, private citizens, or other federal or international agencies (4).

We describe lessons learned from outbreak investigations that involved biologic agents with potential for bioterrorism. In addition, we review investigations in which intentional contamination was considered as a potential cause of the outbreak.

Methods

A standardized form was used to collect data from each investigation from 1988 to 1999. Trip reports submitted by EIS officers after the investigations served as primary sources of information. We focused on outbreaks caused by biologic agents with high potential for bioterrorism, such as *B. anthracis*, *Yersinia pestis*, *Francisella tularensis*, variola virus, viral hemorrhagic fever viruses, *Clostridium botulinum* toxin, *Vibrio cholerae*, *Rickettsia rickettsiae*, encephalitis viruses, *Brucella* species, *Burkholderia mallei* and *B. pseudomallei*, and others according to our preparedness plans (2). We also identified outbreaks in which bioterrorism or intentional contamination was considered. Because each outbreak represented possible bioterrorism, we examined outbreaks in which the etiologic agent remained unidentified. From the trip reports, we abstracted information on possible bioterrorism, causative agent, location, time from first case to first report of the outbreak, and source of recognition and reporting of the outbreak.

We defined the source of recognition and reporting as the person, persons, or institution that originally brought the outbreak or health emergency to the attention of health authorities, as recorded in the trip report. While diagnosis and reporting may be ongoing during an investigation, the initial recognition of an outbreak is a singular event that can occur at the peripheral or primary care setting or at the local, state, or federal level.

We defined the beginning of the outbreak as onset of illness in the first case of the outbreak cluster. Diagnosis of the first illness in an outbreak may occur before the epi-

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demic is recognized and is often determined retrospectively. Epi Info 6 software (CDC, Atlanta, GA) was used to enter the data from the abstractions of the trip reports. SAS software, release 6.12, (SAS Institute Inc., Cary, NC) was used to generate descriptive statistics.

Results

Several agents have been identified as likely to be used in bioterrorism (2). Of the 1,099 investigated outbreaks, 44 (4.0%) were caused by an agent with potential for bioterrorism (Table 1). *V. cholerae* was responsible for 18 outbreaks, *Y. pestis* for 11, viral hemorrhagic fever for 7, *Bacillus anthracis* for 3, and *C. botulinum* toxin for 3. *F. tularensis* and *R. rickettsiae* accounted for one outbreak investigation each. The causative agent was not identified in 41 (3.7%) investigations.

The 44 outbreaks involving an agent with potential for bioterrorism and the 41 caused by unknown infectious agents are summarized by location, year, disease agent, and conclusion (Table 2). All botulism outbreaks (two in the United States) were linked to contaminated food. Ten of the 11 plague outbreaks occurred in U.S. areas of known endemic plague in animals. Of the 18 cholera investigations, 4 were in the United States and involved nursing home patients, imported food, raw fish, and contaminated food on an international flight. Twelve (29%) of the 41 outbreaks caused by unknown agents involved cruise ships.

Intentional use of infectious agents to cause harm to civilians (i.e., bioterrorism) was considered in six investigations (Table 3) (5–8). Although the event did not occur during the period of this review, we included an outbreak of salmonellosis associated with contamination of a salad bar in Oregon in 1984. Several years after the investigation, contamination was (during the study period) determined to be intentional.

Healthcare providers were the source of 270 (24.6%) reports, and infection control practitioners were the source of 129 (11.7%). Together, these two categories of healthcare professionals were the most common source of outbreak recognition and reporting, accounting for 399 (36.3%) reports. Health departments accounted for 335 (30.5%) reports. Some of these 335 outbreaks may have been originally reported to local health departments by clinicians or clinical laboratories, but the original reporting source may have been missing from the trip report. Other sources of recognition and reporting of these outbreaks were existing surveillance systems (55, 5.0%), foreign ministries of health (30, 2.7%), nongovernmental organizations (22, 2.0%), the World Health Organization (16, 1.5%), and the Indian Health Service (12, 1.1%). Forty-nine (4.5%) outbreaks were reported by other sources, such as private clinics, laboratories, or private citizens. More than one reporting source was found in 58 (5.3%) cases. In 123 (11.2%) outbreaks, no mention was made of the recognition and reporting source, the method of recognition and reporting was unclear, or both the source and the method of recognition and reporting were unclear.

Table 1. Epidemic Intelligence Service field investigations involving unknown agents and potential agents of bioterrorism, 1988–1999

Agent	Frequency	% of investigations (n = 1,099)
Unknown infectious agent[a]	41	3.7
Vibrio cholerae	18	1.6
Yersinia pestis	11	1.0
Viral hemorrhagic fever virus	7	0.6
Bacillus anthracis	3	0.3
Clostridium botulinum	3	0.3
Coxiella burnetii	1	0.1
Francisella tularensis	1	0.1
Total	85	7.7

[a]In these cases, the outbreak was considered to be caused by an infectious agent because of the characteristics of the illness and outbreak.

The number of days from the beginning of the outbreak to the date the problem was first identified by the agency requesting CDC assistance was 0 to 26 days (Table 4). The time from the date the initial patient became ill to the date the initial contact was made to the requesting agency for the unexplained critical illness investigation was 26 days (Epi-Aid 99-59). The number of days from the date the problem was identified by the requesting agency to the date of initial CDC contact was 0 to 6 days.

Discussion

Investigations from 1988 to 1999 included outbreaks caused by *B. anthracis* spores, *V. cholerae, Y. pestis, F. tularensis*, *Coxiella burnetii*, Venezuelan equine encephalitis virus, viral hemorrhagic fever virus, and *Clostridium botulinum*; all of these agents might pose a bioterrorism threat, were responsible for 4% of all outbreaks from 1988 to 1999, and are not common causes of outbreaks investigated by CDC. A single case of illness or death caused by any of these organisms should suggest intentional exposure (or accidental exposure in which the perpetrators inadvertently exposed themselves to the causative agent.)

However, not all bioterrorism has involved or will involve these high-threat (formerly identified as weaponized) agents. In 1997, a laboratory worker intentionally contaminated his co-workers' food with a strain of *Shigella* stolen from the laboratory (9). While the *Shigella* strain did cause severe gastroenteritis and several hospitalizations, the use of this strain deviates from the popular idea of a bioterrorist's preferred weapon. However, viewing the bioterrorist's preferred weapon as a high-threat, aerosolizable, infectious agent that may cause immediate, widespread outbreaks may mislead preparedness efforts.

Table 2. Trip reports, involving unknown infectious agents or potential agents of bioterrorism (ultimately not considered bioterrorism), the Centers for Disease Control and Prevention, January 1988–December 1999

Report no.	Y	Location	Etiologic/agent	Conclusion
90-56	1990	Texas, USA	Unknown	Rash and fever in children, no discernable cause
93-02	1992	Wyoming, USA	*Coxiella burnetii*	Q fever in two bentonite miners
94-02	1993	Georgia, USA	*Clostridium botulinum*	Botulism outbreak linked to contaminated food
94-32	1994	Five states, USA	Unknown	Cluster of cases, no discernable cause
94-42	1994	Texas, USA	*C. botulinum*	Botulism outbreak linked to contaminated food
94-86	1994	Connecticut, USA	Sabia virus	Accidental infection with Sabia virus in laboratory worker
94-88	1994	Bolivia	Machupo virus	Bolivian hemorrhagic fever outbreak
95-16	1994	Utah, USA	Unknown	Contaminated solution used in grafting procedure; source undefined
95-40	1995	Palau	Dengue type 4 virus	Dengue type 4 virus outbreak
95-55	1995	Kikwit, Zaire	Ebola virus	Ebola hemorrhagic fever outbreak
95-61	1995	South Dakota, USA	*Francisella tularensis*	Tick-borne tularemia
98-23	1998	Kenya; Somalia	Rift Valley fever virus	Rift Valley fever outbreak
98-28	1998	Argentina	*C. botulinum* toxin	Botulism outbreak linked to contaminated food
98-35	1998	Uganda	Rift Valley fever virus	Rift Valley fever virus outbreak
98-55	1998	Texas, USA	*Bacillus anthracis*	Exposure to live spore vaccine for anthrax
98-83	1998	Kazakhstan	*B. anthracis*	Reemergence of anthrax, Kazakhstan
11 investigations involving plague	Multiple	10 investigations in USA (Oklahoma, Arizona, New Mexico, Texas, California); one in India.	*Yersinia pestis*	Mostly in areas of endemic plague in animals
18 investigations involving cholera	Multiple	4 investigations in USA (Mississippi, Maryland, Hawaii, California), 14 elsewhere	*Vibrio cholerae*	Cholera in two nursing home patients, outbreak involving imported food, outbreak involving consumption of raw fish, and outbreak involving contaminated food on international flight
12 investigations involving unknown agent on cruise ships	Multiple	Cruise ships	Unknown	Gastroenteritis outbreaks in which infectious agent was not identified by laboratory testing
26 additional investigations involving unknown agent	Multiple	24 in USA, 2 elsewhere	Unknown	Gastroenteritis outbreaks, acute illness after surgical procedures, and other outbreaks in which no infectious agent was identified by laboratory testing

In 1984, the outbreak of salmonellosis associated with intentional contamination of a salad bar in Oregon was not initially considered intentional (8); however, further investigation proved that it was. Intentional contamination may resemble naturally occurring outbreaks, may spread slowly through a population, and may involve endemic pathogens. Because of the potential similarity between naturally occurring and intentional outbreaks and the increased threat of bioterrorism in the United States, the index of suspicion for intentional exposure should be high.

Despite advances in the identification of pathogens, outbreaks of unexplained illnesses continue to occur. In this review, we found 41 outbreaks in which the causative agent remained undetermined. Intentional contamination should be considered in these cases because 1) unusual or not easily explained outbreaks are more likely to be caused by intentional contamination, 2) outbreaks resulting from bioengineered pathogens may have unusual or unexpected characteristics, and 3) bioengineered pathogens may not be easily detected by existing assays. For these reasons, outbreaks with unexpected or unusual clinical or epidemiologic characteristics should be pursued with added urgency, and investigators should consider the possibility of previously unidentified or newly engineered pathogens.

While CDC is often notified about outbreak investigations by a state or national health department, the origins of these reports are diverse and include local health departments, surveillance systems, physicians, veterinarians, infection control practitioners, organizations (e.g., the U.S. Food and Drug Administration or the World Health Organization), laboratories, private citizens, ship doctors, vessel sanitation programs, and others. We found that physicians and infection control practitioners reported more than one third of outbreaks. This estimate is probably

Table 3. Epidemic Intelligence Service investigations in which bioterrorism or intentional contamination was considered a cause

Report No.	Outbreak	Conclusion
84-093	Salmonellosis, Oregon, 1984	A total of 751 persons became ill with salmonella gastroenteritis. Religious group deliberately contaminated salad bars. *Salmonella enterica* Typhimurium strain found in laboratory at commune was indistinguishable from outbreak strain (5).
97-008	*Shigella dysenteriae* type 2, Texas, 1996	Diarrheal illness in hospital laboratory workers who ate pastries, anonymously placed in break room. Identical strains of *S. dysenteriae* type 2 were isolated from stool cultures of case patients, from recovered muffin, and from laboratory stock culture, part of which was missing.
98-006	*S. sonnei*, New Hampshire, 1997	Seven laboratory workers at local hospital became ill with gastroenteritis. Most cases caused by strain of *S. sonnei* that was highly related to a stock culture strain maintained by the hospital laboratory. Possibility that first two cases were caused by intentional contamination could not be excluded.
99-025	Anthrax hoaxes, 1998	Centers for Disease Control and Prevention received reports of alleged anthrax exposure; letters were sent to health clinics in Indiana, Kentucky, and Tennessee and to private business in Tennessee; three telephone threats of anthrax contamination of ventilation systems were made to public and private buildings; all threats were hoaxes.
99-059	Unexplained critical illness, New Hampshire, 1999	A 38-year-old woman was admitted to a hospital with fever, myalgia, and weakness; severe illness and death occurred 32 days after hospital admission; serum specimens indicated *Brucella* species. Patient's history of multiple febrile illnesses suggested unspecified autoimmune process.
99-94-1	Encephalitis cluster, New York City, 1999	Several residents were hospitalized with illness of unknown etiology characterized by fever, encephalitis, axonal neuropathy, and flaccid paralysis (unpublished data: Epi-1 report); increase in deaths of New York City birds, especially crows; human and bird tissue samples were positive for West Nile–like virus.

low because the reports that were recorded as originating from local or state health departments may actually have been brought to the attention of health departments by frontline practioners. Because of the importance of this frontline in detection and reporting, preparedness efforts must include education and support of these healthcare professionals. The clinical laboratory should have the capacity and legal latitude to use all appropriate testing. This capacity should include Gram stain of tissue smears for agents such as *B. anthracis*.

Trip reports (Epi-2) are summaries, not finalized data and are written for the state and local health departments and CDC and the U.S. Department of Health and Human Services. They are primarily internal documents and are not independently peer reviewed or standardized; however, each investigation may use standardized techniques. In general, problems we encountered were not inaccuracies (when a subset of trip reports were compared to the articles that followed them) but rather incompleteness of data we were interested in reviewing. We suggest that trip reports include standardized data collection on certain variables important in evaluating the effectiveness of detecting and reporting outbreaks (e.g., source of outbreak detection, date of the first case diagnosis, and date the outbreak was recognized).

Because we cannot rely on astute healthcare practitioners alone, existing national health surveillance systems should be modified or strengthened to increase their effectiveness in identifying bioterrorism (10). Systems already in existence for laboratory-based reporting should be enhanced for use in bioterrorism surveillance. Improved surveillance for unexplained critical illness and death may also be an important component in improved health surveillance for bioterrorism (5).

In addition to healthcare providers and public health departments, other persons and organizations may identify and report outbreaks. For example, veterinarians may be the first to see evidence of bioterrorism because pets and livestock may be more susceptible than humans to agents released in the environment or because a large susceptible

Table 4. Number of days from beginning to notification for outbreaks in which bioterrorism or intentional contamination was considered

Report no.	Investigation	Beginning of outbreak	No. of days from first case to problem identification	No. of days from problem identification to initial CDC contact
84-93	Large salmonellosis outbreak caused by intentional contamination of restaurant salad bars, Oregon	9/15/84	6	4
97-008	Shigellosis outbreak in hospital laboratory workers, Texas	10/29/96	1	1
98-006	*Shigella sonnei* outbreak in laboratory workers, New Hampshire	9/20/97	17	3
99-25	Anthrax hoaxes	10/30/98	0	0
99-59	Unexplained critical illness, New Hampshire	3/24/99	26	1
99-094	Encephalitis cluster with paralysis of unknown etiology, New York (West Nile virus)	8/9/99	14	6

[a]CDC, Centers for Disease Control and Prevention.

animal population may be present in the affected area (11). Detection of disease in lower animals may be essential to detecting a bioterrorism event because most of the bioterrorism threat agents are zoonotic disease agents, causing disease in both humans and lower animals. The West Nile virus outbreak, while naturally occurring, is a good example of the importance of animal disease surveillance because detection of illness and death in birds was important to identification of the outbreak.

Other potential resources include persons not in the healthcare field. Employers may notice a high rate of illness in their employees, or schools may report a larger than usual absentee rate. Enhancing surveillance systems, providing a mechanism of instant reporting to the proper officials, educating healthcare professionals and others in the community, and strengthening knowledge and skills for thorough outbreak investigations will improve collective preparedness for bioterrorism. In the future, shortening the time from detecting to reporting an outbreak to public health authorities, including CDC, will be essential to an effective response. National health surveillance systems are an important adjunct that, with further development, may allow for early detection of bioterrorism. Finally, education about bioterrorism should go beyond a mere description of the threat agents and strive to enhance the epidemiologic and investigative skills of healthcare professionals, including laboratory personnel, especially those in primary care settings, who are likely to be the first contact for people and communities affected by acts of bioterrorism.

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Endemic Gastrointestinal Anthrax in 1960s Lebanon: Clinical Manifestations and Surgical Findings

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Anthrax is an ancient disease caused by the gram-positive *Bacillus anthracis*; recently, it has gained much attention because of its potential use in biologic warfare. Anthrax infection occurs in three forms: cutaneous, inhalational, and gastrointestinal. The last type results from ingestion of poorly cooked contaminated meat. Intestinal anthrax was widely known in Lebanon in the 1960s, when a series of >100 cases were observed in the Bekaa Valley. We describe some of these cases, introduce the concept of the surgical management of advanced intestinal anthrax, and describe some of the approaches for treatment.

Several reports and reviews have recently shed light on anthrax and its cutaneous and pulmonary manifestations, focusing on its threat as a biologic weapon. The third form of the disease, gastrointestinal anthrax, has not received as much attention, and the research describing its manifestations is scarce. We report on several cases of intestinal anthrax from 1960 to 1974 in the Bekaa Valley in Lebanon, where the consumption of raw or poorly cooked meat is customary. One of the authors (A. Ghossain) treated and operated on all the patients described here. We describe a series of gastrointestinal anthrax cases, the reporting of which has become paramount with the current renewed interest in this entity, the scarcity of information in the research on this particular form of anthrax, and the high fatality rate in advanced disease. The cases described in this report were chosen to illustrate the protean manifestations of gastrointestinal anthrax.

Clinical Manifestations and Surgical Findings

In March 1960, an acute and particularly severe abdominal syndrome was recognized in the Bekaa Valley in Lebanon. The first cases were described in male patients from 4 to 25 years of age with an illness that consisted of three phases. Phase I was marked by fainting spells, asthenia, low-grade fever, and headache. Patients rarely sought medical treatment at this stage because the symptoms were not serious enough. In the few patients seen at this stage, physical examination disclosed flushing of the face and red conjunctivae, but appearance was not otherwise affected. The first impression of a general practitioner who saw patients during this phase of illness was that the patient had an early viral infection. Phase II started when, 24 hours later, early symptoms persisted, and abdominal pain of variable intensity supervened. The pain description ranged from mild to severe paroxysms to constant pain. Low-grade fever, nausea, and vomiting were frequent; diarrhea, if present, was mild. Physical examination usually showed a smooth, ill-defined mass in the right lower quadrant or the periumbilical area and abdominal distention. At that stage, patients were referred to a specialist for acute abdominal infection. Three clinical findings, however, did not fit this diagnosis: 1) the illness started with vague, generalized symptoms instead of abdominal pain; 2) evidence of ascites was found on examination, 3) and patients had severe weakness and intravascular depletion, findings uncommon in early appendicitis.

Phase III was recognized because most patients were referred at that stage with rapid increase in abdominal girth and paroxysms of abdominal pain. Occasionally, gastrointestinal bleeding concurred. Upon examination, some features were frequently present: shock, ascites, flushed face, and red conjunctivae. Because of unclear and questionable diagnosis, exploratory laparotomy was performed on several patients, invariably showing an abundant yellowish and thick ascitic fluid, soft hypertrophied mesenteric lymph nodes (3 cm–5 cm) mostly in the ileocecal region, and substantial edema involving one segment of small bowel, cecum, or ascending colon. When the diseased segment was not resected during surgery, many patients experienced a rapid but transient postoperative clinical improvement. Ascites, however, soon reaccumulated, and most of these patients died from an overwhelming state of shock. Definite and steady recovery was observed in most patients who underwent partial bowel resection. Examination of the bowel segment at surgery or autopsy disclosed a central necrotic lesion (2 cm–3 cm) encircled by small soft red nodules (0.5 cm–1 cm in diameter), and surrounded by severe edema of the bowel wall with areas

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of hemorrhage. Bowel perforation was uncommon, as patients usually died before that progression (1,2).

Most of the patient population had these signs and symptoms. In some cases, however, two unusual clinical pictures were observed: the surgical form and abortive form. In the surgical form, the illness started with severe abdominal pain without the generalized symptoms of phase I. Surgery was often performed with the provisional diagnosis of acute infection in the abdomen. In the abortive form, the illness was limited to phase I, probably because such patients had taken penicillin or other antibiotics, thus interrupting the cycle of the disease (2,3).

Epidemiologic Investigation

More cases were being described, but no clear diagnosis could be made until October 1960, when the cause of this curious clinical syndrome was elucidated. A 30-year-old woman with abdominal symptoms and ascites underwent laparotomy but died 12 hours later. Surgery showed ascites, an edematous small bowel loop, and huge mesenteric lymph nodes. Intestinal resection and lymph node biopsies were performed. Pathologic examination showed a central mucosal necrotic lesion along with hemorrhage and extensive edema. Gram-positive bacilli in chains were also observed. Bacterial cultures from blood and excised lymph nodes grew *Bacillus anthracis*. Inoculation of test animals resulted in death within 20 hours; their spleens had large amounts of anthrax bacilli (4). The identity of the bacillus was further confirmed when tissue specimens were sent to the Centers for Disease Control and Prevention (CDC) for analysis.

With this new information at hand, investigations were conducted to pinpoint the source of infection. The patient's husband, a shepherd, was questioned and admitted that 4 days before the onset of his wife's illness, he had slaughtered a dying goat. The meat was consumed raw by friends and family members (customary in some remote villages). All persons who ate meat were identified. The patient's sister-in-law had had cutaneous anthrax above the lip and had an uneventful course with penicillin therapy. Another person had complained of vague abdominal pain and mild diarrhea; stool culture was negative. The goat's skin was examined and found to have anthrax spores. Medical records of previous patients were then reviewed; their families were contacted, and a number of persons did recall eating raw meat few days before symptoms began. Furthermore, paraffin-embedded tissue blocs from a patient who died earlier of a similar illness were reexamined; evidence of gram-positive bacilli was found in the intestinal tissue (4).

Over 100 cases of intestinal anthrax were subsequently observed during a period of 14 years. Most patients were shepherds or their relatives who lived in remote villages of the Bekaa Valley. Their livestock was not vaccinated against anthrax, and they regularly lost some of their herds to a sickness they called "the disease of the spleen" because that organ was always massively swollen. Sick animals were slaughtered; the skin was sold, and meat was often ingested raw or inadequately cooked. The liver was usually eaten by children and young adults.

Clinical Cases

Case 1

In August 1961, a 26-year-old man complained of abdominal pain, nausea, vomiting, and generalized weakness of 4 days' duration. Upon admission to the hospital, he was in a state of shock but was afebrile. He had a history of ingestion of raw meat 10 days before the symptoms began. On physical examination, he had abdominal tenderness and evidence of ascites. Analysis of the ascitic fluid showed an albumin level of 6.0 g/L and gram-positive rods with central spores. Cultures from the fluid grew *B. anthracis*. Attempts at resuscitation with intravenous fluids and treatment with penicillin failed, and the patient died 21 hours after admission.

Case 2

A 24-year-old shepherd was admitted to the hospital in September 1961 with severe nausea, vomiting, and abdominal pain. His illness had started 1 week earlier with headache and mild abdominal pain. He reported eating raw meat from an ill goat. His younger brother, who ate from the same goat meat, had died of intestinal anthrax on the same day of admission as this patient. His pulse was 120 bpm, systolic blood pressure 95 mm Hg, and temperature 37.5°C. The patient had erythema and edema of the face, and a healing eschar on the lower lip that had reportedly started as a small vesicle. He had diffuse abdominal tenderness. His leukocyte count was 12,500 cells/mm^3, with 73% neutrophils. Blood cultures grew *B. anthracis*. The patient received intravenous penicillin and improved. He was discharged 1 week later in good condition.

Case 3

A 7-year-old boy was admitted in July 1965 with periumbilical pain, vomiting, and low-grade fever. He had eaten raw meat from an ill goat. His abdomen was distended, with a palpable mass in the right iliac fossa and evidence of ascites. His systolic blood pressure was 80 mm Hg. His leukocyte count was 13,000 cells/mm^3, with 86% neutrophils. The presumptive diagnosis was acute appendicitis. He underwent laparotomy, which yielded approximately 2 L of fluid. The cecum and ascending colon were edematous, with a hemorrhagic mucosa; mesenteric lymphadenopathy was noted. Two days earlier, his mother

reported a skin lesion over the left eyelid and severe surrounding edema suggestive of cutaneous anthrax. The patient was treated with penicillin but had persistent high-grade fever until day 9 of admission, when he was no longer febrile and was then discharged in good condition. In this particular case, the clinical and surgical findings suggested intestinal anthrax, although no microbiologic evidence was obtained.

Case 4

A 17-year-old man was admitted to the hospital in October 1962 with abdominal pain, generalized weakness, and high-grade fever; he had eaten raw meat. On examination, the abdomen was distended with ascites, with tenderness in the right lower quadrant. The patient underwent laparotomy for suspected acute appendicitis that showed edema of the cecum and ascending colon, as well as enlargement of the mesenteric lymph nodes (Figure 1). Lymph node tissue, as well as blood and ascitic fluid cultures, grew *B. anthracis*. Gram-positive rods were visible upon pathologic examination of the excised lymph nodes (Figure 2). After surgery, the patient received penicillin and streptomycin. He improved slowly and was discharged on day 18.

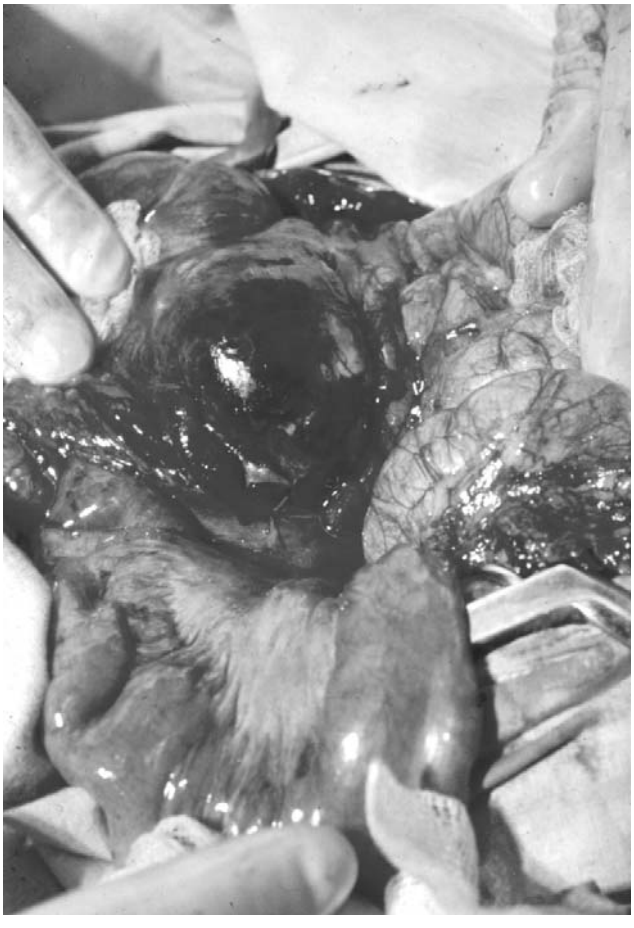

Figure 1. Extensive edema and hemorrhage involving the cecum in a patient with intestinal anthrax.

Case 5

In March 1974, a 15-year-old boy arrived at the hospital in shock. He had ascites and oculofacial congestion. Five days earlier, he had consumed raw meat from a sick goat. Despite aggressive resuscitation and treatment with penicillin, he remained in critical condition. Exploratory laparotomy revealed extensive edema of the cecum with mesenteric lymphadenopathy. Right hemicolectomy with primary anastomosis was performed along with continuous closed peritoneal drainage. His condition improved dramatically, and he was discharged 10 days later. Cultures from the mesenteric lymph nodes grew *B. anthracis*.

Case 6

A 20-year-old woman was admitted to the hospital in September 1974 with a 2-day history of abdominal pain after eating poorly cooked meat from a dying goat a week earlier. She was in a state of shock and had abdominal distention and a doughy mass in the periumbilical area. Laparotomy showed a large amount of ascitic fluid, an edematous small bowel loop proximal to the cecum, and enlarged mesenteric lymph nodes (Figure 3A and B). Intestinal resection and continuous drainage of the ascites were performed. Cultures from intraoperative lymph node samples grew *B. anthracis*. The patient improved gradually and was discharged 12 days later.

Discussion

Gastrointestinal anthrax is extremely rare in the United States and western Europe but is more frequently encountered in developing countries around the globe. Research describing the clinical manifestations of this entity is scarce, poorly detailed, and inaccurate about appropriate management (5).

We performed a MEDLINE search to evaluate data published on gastrointestinal anthrax. Research that could be accessed and reviewed consisted of 11 reports from 1970 to 2000, most of which described single cases. Most patients were from developing countries, namely Iran, Bangladesh, Zimbabwe, Thailand, Uganda, India, and Turkey (6–15) (Table). Only two cases were reported from the United States, by CDC in 2000 (16). The infection was uniformly associated with eating contaminated meat, although theoretically, any ingested item could act as a vehicle for the transmission of anthrax spores. One study by Ndyabahinduka et al. was of an epidemic of gastrointestinal anthrax in Uganda in 1984, which affected 143 of 155 persons who ate meat from an infected zebu (Asian ox). In most cases, symptoms were those of gastroenteritis, with abdominal tenderness, vomiting, and diarrhea. Three

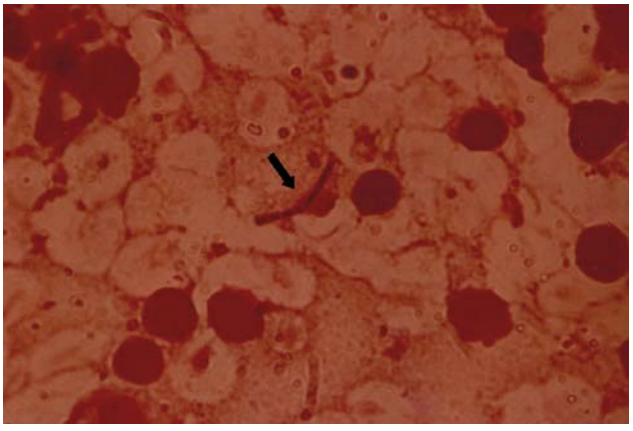

Figure 2. Anthrax bacilli (arrow) within mesenteric lymph node tissue.

children had blood-tinged stools from which anthrax bacilli were isolated. Thirteen patients had pharyngeal edema of variable severity. A fatal outcome from fulminant gastroenteritis was reported in nine patients, all children. All other patients responded quickly to tetracycline or penicillin (10). Another study by Phonboon et al. described an outbreak of gastrointestinal anthrax after an outbreak in cattle in Thailand; 74 persons become ill, and 3 died (9).

In published cases of gastrointestinal anthrax, death was more common in patients who had severe symptoms, including hematemesis, vomiting, abdominal pain, and distention (phase III), and who were only treated with antibiotics (6,11,13,14). Surgical exploration and bowel resection was performed in two patients first seen in phase III (8,15). The disease involved the ascending colon alone in one case and the cecum and ascending colon in the other. After surgery and antibiotic therapy, both patients recovered and were discharged. These two cases illustrate the benefit of surgery in the advanced form of gastrointestinal anthrax. These findings support our current approach for managing patients whose condition remains unstable after 6 to 12 hours of treatment, namely, administering antibiotics and adequate resuscitation and then resecting the diseased bowel segment. The rationale behind surgical resection is to overcome not only the large bacterial load but also the larger load of toxin in diseased tissues. In all our cases, the disease was confined to a single bowel segment, mostly small bowel or cecum. The clinical condition of patients improved rapidly and steadily after resection. Initial results were disappointing, since several patients died postoperatively of anastomotic leaks, dehiscence, and fistulization. Incomplete resection, severe hypoproteinemia, and rapid reaccumulation of protein-rich ascitic fluid were the main causes of surgical failure.

Based on our experience, the approach used in the management of cases of gastrointestinal anthrax should consist of: 1) initiation of intensive intravenous antibiotic therapy as soon as the diagnosis is made, 2) wide resection into seemingly healthy tissues with primary anastomosis in patients who did not improve with medical therapy, 3) continuous drainage of the ascites, as fluid will continue to accumulate for several days after surgery, 4) and aggressive replacement of protein and electrolyte losses (2,17). However, to make any generalization about the preferred mode of treatment of gastrointestinal anthrax in the absence of solid and reproducible clinical and epidemiologic data would be difficult. Furthermore, with the current improved access to medical care and advances in diagnostic techniques and supportive measures compared to the 1960s, surgical intervention might now be limited to few cases of advanced disease unresponsive to medical therapy.

Following the 1960s outbreak, some areas were recognized as being contaminated by anthrax spores. Grazing of livestock in these "damned fields" (as they were called by ancient French farmers) has since been avoided by shepherds, thereby virtually eliminating the disease from the Bekaa Valley (2,3).

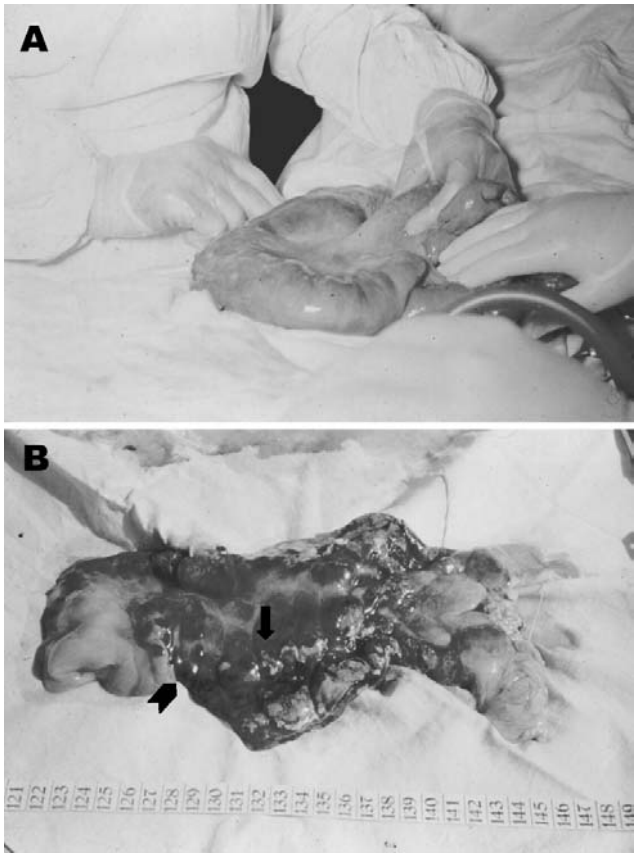

Figure 3. A. Severe edema of a small bowel loop in intestinal anthrax with a large mesenteric lymph node held between the surgeon's fingers. B. Same segment of bowel opened after resection. Edema, necrosis, and mucosal hemorrhages exist. A central eschar (arrow) and small surrounding nodules (arrowhead) are reminiscent of the cutaneous lesions of anthrax.

Table. Reports of gastrointestinal anthrax published from 1970 to 2000

Y	Authors (Reference)	Country	No. of patients	Disease location	Treatment	Outcome
1970	Dutz et al. (7)	Iran	1	Stomach	Antibiotics	Died
1977	Nalin et al. (8)	Bangladesh	1	Unspecified	Antibiotics	Survived
1980	Jena (9)	Zimbabwe	1	Ascending colon	Surgery	Survived
1984	Phonboon et al. (10)	Thailand	74	Unspecified	Unspecified	3 died
1984	Ndyabahinduka et al. (11)	Uganda	143	Unspecified	Antibiotics	9 died
1985	Bhat et al. (12)	India	1	Unspecified	Antibiotics	Died
1990	Sekhar et al. (13)	India	20 internal*	Unspecified	Antibiotics	Unspecified
1990	Kunanusont et al. (14)	Thailand	3	Stomach	Antibiotics	1 died
1995	Alizad et al. (15)	Iran	1	Unspecified	Antibiotics	Died
1997	Tekin et al. (16)	Turkey	1	Cecum and ascending colon	Surgery	Survived
2000	CDC (17)	USA	2	Unspecified	Antibiotics	Survived

[a]Internal includes inhalational anthrax, gastrointestinal anthrax, anthrax meningitis, and septicemia; CDC, Centers for Disease Control and Prevention.

Conclusion

We describe the clinical spectrum of gastrointestinal anthrax, a disease that was endemic in Lebanon in the 1960s. We also report on the success of surgical treatment in some of the advanced cases, emphasizing the vital role of aggressive supportive measures in patient management. Our report lacks detailed epidemiologic data describing the incidence, age and sex distribution, and outcome of patients because most cases date back to the 1960s, a time when epidemiologic studies were scarce in a developing country such as Lebanon. Our experience with gastrointestinal anthrax, however, remains valuable because of the rarity of this condition and the dearth of data on management approaches.

Naturally, the consumption of raw meat, still widely practiced in many countries, should be strongly discouraged through education of the mass population as to the health hazards associated with such a custom. Ultimately, however, the control of anthrax in humans is chiefly dependent on control of the disease in animals. In the event of an anthrax enzootic, extensive investigations should be conducted to identify the source of infection and eliminate it. Milk from areas experiencing enzootics should be discarded, and sick animals should be isolated. Affected farms should be quarantined for at least 2 weeks after the last death from anthrax. The release of *B. anthracis* in the environment should be avoided to prevent future disease; dead animals should be burned or buried deeply and covered with lime. Their carcasses should not be slaughtered or necropsied, as exposure of the vegetative forms to the ambient atmosphere enhances sporulation. Annual vaccination of livestock and avoidance of clandestine slaughtering remain vital to the prevention of disease in endemic areas (18–20).

Dr. Kanafani has completed residency training in Internal Medicine at the American University of Beirut Medical Center and is currently pursuing a master's degree in epidemiology at the American University of Beirut. She is interested in further studying the epidemiology of *Bacillus anthracis* in other areas of the Lebanese territory. Her research interests also include nosocomial infections and infective endocarditis.

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Vero Cytotoxin–Producing *Escherichia coli* O157 Gastroenteritis in Farm Visitors, North Wales

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An outbreak of Vero cytotoxin–producing *Escherichia coli* O157 (VTEC O157) gastroenteritis in visitors to an open farm in North Wales resulted in 17 primary and 7 secondary cases of illness. *E. coli* O157 Vero cytotoxin type 2, phage type 2 was isolated from 23 human cases and environmental animal fecal samples. A case-control study of 16 primary case-patients and 36 controls (all children) showed a significant association with attendance on the 2nd day of a festival, eating ice cream or cotton candy (candy floss), and contact with cows or goats. On multivariable analysis, only the association between illness and ice cream (odds ratio [OR]=11.99, 95% confidence interval [CI] 1.04 to 137.76) and cotton candy (OR=51.90, 95% CI 2.77 to 970.67) remained significant. In addition to supervised handwashing, we recommend that foods on open farms only be eaten in dedicated clean areas and that sticky foods be discouraged.

Human illness caused by Vero cytotoxin–producing strains of *Escherichia coli* O157 (VTEC O157) can occur after direct contact with farm animals. Although the annual rate of VTEC O157–reported illness in the general population in England and Wales is relatively low, ranging from 1.28 to 2.10 /100,000/year from 1995 to 1998 (1), young children who become ill are at particular risk for serious complications, such as hemolytic uremic syndrome.

An estimated half million to 10 million visits each year are made to approximately 1,000 open farms (i.e., a working farm that allows visitors, usually for an entry fee) in England and Wales (2; Association of Farms for Schools, pers. comm.). However, only occasional outbreaks of VTEC O157 associated with such visits are reported: eight outbreaks of VTEC O157 in visitors to open farms in England and Wales were reported to the Laboratory for Enteric Pathogens at Colindale in 1992 through 2000 (3–7). The largest outbreak in the United Kingdom during this period consisted of seven cases (5). Individual cases associated with open farms are rarely reported (8). Of 71 reported cases of *E. coli* O157 in Wales during 1998, two primary case-patients reported visiting an open farm in the previous week (Communicable Disease Surveillance Centre, Wales, unpub. data).

Recognition of the risk of acquiring zoonotic infection, particularly gastrointestinal illness, has led to written guidelines for open farms (9). The guidelines concentrate on farm layout, clear routes for visitors to follow, staff training, control of animal contact, separate eating areas, adequate handwashing facilities, and adequate information in the form of notices or leaflets. These recommendations are derived from an understanding of how pathogens are likely to be spread to humans. However, the popularity of farms as a tourist attraction, when compared with the infrequency of illness, suggests that quite specific risks may occasionally occur. The opportunities for studying these risk factors more systematically are limited, as outbreaks are so infrequent. A large outbreak of gastroenteritis in North Wales, associated with VTEC O157, presented an opportunity to conduct a case-control study to investigate

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[1]C. Payne designed the case-control study, drew up the questionnaire, and analyzed and wrote up the results. M. Petrovic helped design the questionnaire, organized the data collection, and wrote the sections describing the outbreak and microbiologic findings. R.J. Roberts contributed toward the study and questionnaire design, including devising the method of selecting controls; he also helped in the final drafts of the paper. R.L. Salmon originally conceived the study, commented on the questionnaire design, and helped interpret the findings. T. Cheasty was responsible for confirmation and typing of isolates. G. Willshaw performed DNA-based comparison of strains. Other authors made important contributions to the investigation and control of the outbreak and participated in writing the paper.

which factors were associated with an increased risk for illness.

Identification, Investigation, and Control of the Outbreak

On June 9, 1999, the first report of *E. coli* infection in a person who had visited an open farm in North Wales was made to the communicable disease control team of North Wales Health Authority. The farm was visited and found to be operating at a generally high standard. Six days later, on June 15, two more patients with culture-positive *E. coli* O157 infection reported having visited the farm. All three case-patients had visited the farm on May 31. The farm owner immediately and voluntarily closed the farm, and all local physicians were informed of the cases by fax on June 15 and asked to report further cases.

The farm received 50,000 visitors a year and had a range of animals and several food outlets. Most contact with animals occurred in the barn, which contained a variety of farm and domestic animals. Handwashing facilities existed nearby but were not located immediately adjacent to the barn exits. Eating areas were accessible to a roaming goat. The first three case-patients had visited the farm on May 31, the 2nd day of a 2-day annual festival held on the farm. The festival had admitted 3,000 visitors each day, all of whom had access to the open farm. Attractions at the festival included food stalls and a number of visiting animals (rare and unusual farm animals brought to the farm for the festival). The working part of the farm had a sheep flock and herd of cattle.

Local case-finding efforts on June 15 did not initially disclose any further cases associated with the farm. The outbreak control team recommended washing and disinfecting all public areas and preventing contact between visitors and farm animals or animal feces. After complying with these recommendations, the farm was allowed to reopen 2 days later on June 17; however, it was to operate under a prohibition notice served under the provisions of the Health and Safety at Work Act 1974, restricting visitors from having direct contact with animals. However, later that same day a fourth case was reported in a person who had visited the farm on June 5, five days after the first three patients. The farm was formally closed to all visitors under the terms of a second prohibition notice. A national public warning was issued, all communicable disease control units were alerted, and a telephone helpline was set up and received over 150 calls. Children at three local nurseries and two primary schools, where primary cases had occurred after group visits, were screened for further cases. Health and safety arrangements were reviewed at the farm (9) and the recommendations of the outbreak control team were implemented; these included a one-way flow through the petting area, positioning of washing facilities immediately adjacent to the exit, exclusion of farm animals from eating areas, and reinforcement of the importance of handwashing. On June 25, the farm was allowed to reopen. Because patients reported a wide variety of activities and contact with animals, a case-control study was conducted to identify particular high-risk exposures.

Methods

A case was defined as hemolytic uremic syndrome or *E. coli* O157 culture-positive diarrhea in any child <15 years of age who had visited the farm on or after May 31 and become ill within 10 days of the visit. Controls were selected from children <15 years old who remained well in the 2 weeks after a farm visit and whose parents had contacted the telephone helpline. Children who had visited before May 31 were excluded. Only one control child was chosen from each family or group. Where appropriate, information was obtained from an adult who had accompanied the children to the farm. Attempts to contact callers were abandoned after three separate unsuccessful day and evening phone calls.

Potential risk factors were identified from preliminary interviews, a site visit, and a risk-assessment exercise conducted by the proprietor after the outbreak was discovered. A structured questionnaire was administered by telephone to patients and controls. Questions included date of visit, contact with animals or surrounding barriers, areas of the farm visited, food consumption, personal behavior (e.g., thumb-sucking), handwashing, use of the toilet, whether soiling was visible on the child, and whether the child had fallen at the farm. In an attempt to categorize the degree of exposure to each type of animal, respondents were asked to estimate the time they spent with each. The nature of the animal contact was recorded as cuddling, kissing, feeding from hand, bottle feeding, or stroking.

The risk for illness, expressed as an odds ratio (OR), was calculated for each exposure, using Epi Info version 6 (Centers for Disease Control and Prevention, Atlanta, GA). Some exposures, such as animal contact, were analyzed both by category ("contact/no contact") and by comparison of the risk for light contact (only feeding from the hand or bottle feeding) with more intense contact (cuddling, stroking, or kissing). To investigate confounding, logistic regression was performed, using SPSS version 7.5 for Windows (Microsoft, Redmond, WA**),** with the probability of becoming ill as the dependent variable and exposures associated with an increased risk for illness, at $p<0.10$, as independent variables.

Strains were confirmed biochemically and serologically as *E. coli* O157 and were phage typed and tested for resistance to antimicrobial agents by methods summarized previously (10). Pulsed-field gel electrophoresis (PFGE) was performed by the method of Willshaw et al. (11).

Results

Overview of Cases

A total of 17 primary cases (1 adult, 16 children) and 7 secondary cases in household contacts (2 adults, 5 children) were ascertained. Ten patients (1 adult, 9 children) required hospital admission, including 3 children with hemolytic uremic syndrome. Ten of the primary case-patients had visited the farm on May 31, Bank Holiday Monday; the remaining seven had visited during the following 15 days. No patient had visited before May 31. Isolates from all the cases except one, where the sample was not submitted for typing, were characterized as phage type (PT) 2, verocytotoxin type (VT) 2, and were resistant to streptomycin, sulphonamides, and tetracycline (SSuT). Secondary transmission was not evident in any of the schools or nurseries screened.

Microbiology

Eleven of 46 (mainly floor) fecal samples taken by veterinarians were positive for *E. coli* O157. Ten strains were PT2, VT2, SSuT, and one strain was PT4, VT2, and sensitive to antimicrobial agents. These 11 strains were from pens or paddocks containing calves, goats, pigs, sheep, and a pony. Rabbit, fowl, and donkey samples were negative. The results of PFGE showed that the human and animal strains of *E. coli* O157 PT2 VT2 were indistinguishable.

Case-Control Study

Sixteen children met the case definition. Questionnaires were completed for 13 of these 16 case-patients and 36 controls. Controls had a mean age of 4.5 years (SD 2.7) compared with the patients' mean age of 5.4 years (SD 3.1); this difference was not significant (p=0.35). The proportion of girls was similar in patients (55.6%) and controls (61.5%). Table 1 shows results of univariable analy-

Table 1. Results of univariable analysis of risks for illness caused by *Escherichia coli* O157 in visitors to an open farm, Wales

	Cases	Controls		Univariable analysis	
Exposure	Exposed/ not exposed	Exposed/ not exposed	Odds ratio	p value	95% CI
Contact with animals					
Cows	11/2	16/20	**6.88**	**0.01**	1.15 to 52.69
Goats (any contact)	13/0	27/9	Undef	0.09	Undef
Goat (high contact)	10/3	17/19	**3.72**	**0.06**	0.75 to 20.70
Rabbit	9/4	20/16	1.80	0.39	0.40 to 8.62
Sheep	10/3	27/9	1.11	1.00	0.21 to 6.48
Pigs	5/7	11/24	1.56	0.72	0.33 to 7.32
Pony	4/9	20/16	0.36	0.12	0.07 to 1.61
Shire horse	4/9	13/23	0.79	1.00	0.16 to 3.64
Areas of farm visited					
Play area	5/7	27/9	0.24	0.07	0.05 to 1.13
Paddock	6/7	28/8	0.24	0.08	0.05 to 1.13
Pony ride	4/9	15/21	0.62	0.49	0.13 to 2.84
Tractor ride	9/4	28/8	0.64	0.71	0.13 to 3.31
Main barn	13/0	35/1	Undef	1.00	Undef
Food consumption and personal behaviors					
Sucks thumb	1/11	6/30	0.45	0.66	0.02 to 4.74
Bites nails	2/10	6/30	1.00	1.00	0.12 to 7.08
Ate any food while on farm	13/0	33/3	Undef	0.56	Undef
Ate immediately after barn	6/6	11/23	2.09	0.31	0.45 to 9.81
Ate food bought on farm	8/5	14/21	2.40	0.18	0.55 to 10.85
Ate ice cream	9/4	14/22	**3.54**	**0.06**	0.77 to 17.19
Ate cotton candy	7/6	4/32	**9.33**	**0.004**	1.69 to 57.10
Bought animal feed	10/3	32/4	0.42	0.36	0.06 to 2.88
Picked up animal feed from floor	5/8	6/30	3.13	0.13	0.61 to 16.29
Ate animal feed	1/12	0/36	Undef	0.26	Undef
Clung to animal barriers	6/3	25/7	0.56	0.66	0.09 to 3.76
Fell over while on farm	1/12	7/29	0.35	0.66	0.01 to 3.43
Washed hands at all	10/3	24/9	1.12	1.00	0.23 to 7.35
Environmental observations					
Wet underfoot	3/10	6/30	1.50	0.68	0.24 to 8.85
Dirty hands, shoes, or clothes	0/13	9/27	0.00	0.09	0.00 to 1.46
Noticed queue for toilets	2/10	3/32	2.13	0.59	0.21 to 19.56
Type of visit					
Family visit	12/1	33/3	0.92	1.00	0.08 to 30.07
Bank Holiday Monday	11/2	13/20	**9.73**	**0.003**	1.38 to 66.32

[a]CI, confidence interval; undef, undefined (when one of the cells contains a zero, defining a confidence interval is not possible). Bold typeface highlights variables with increased odds ratio statistically significant at 90% level.

sis. As all case-patients had been in contact with goats, calculating an OR for "any contact" was not possible. Goat contact was therefore stratified into high- and low-contact categories.

Attendance on Bank Holiday Monday, eating ice cream, eating cotton candy (i.e., "candy floss"), any contact with cows or goats, and high goat contact were all associated with increased risk ($p<0.10$). All case-patients had eaten either cotton candy or ice cream. No link between the risk for illness and duration of contact with cows or goats was found. The main barn was the only area visited by all patients.

Cotton candy was only available on Bank Holiday Monday, a special festival day on which visitors were also more likely to have contact with cows (OR 5.56, $p=0.03$ for those attending on May 31 compared with other days). For these reasons, Bank Holiday Monday was not an independent variable and so was excluded from the multivariable analysis. The results of multivariable analysis for the other four variables are shown in Table 2. The association between illness and eating ice cream or cotton candy increased and remained significant; the magnitude of effect for cow and goat contact was similar to univariate analysis, although neither factor was statistically significant. To check whether Bank Holiday Monday was unique, the analysis was repeated for the 24 Bank Holiday Monday attenders only; results are shown in Table 3. No OR reached statistical significance, reflecting the smaller dataset; however, the magnitude of the independent effect of these variables (as evidenced by ORs) is similar to that for the whole study population, suggesting that the risks were similar on the Bank Holiday Monday to the whole study period.

Table 2. Results of multivariable analysis of significant animal contact and food consumed and risk for illness (49 observations)[a]

	OR	p value	95% CI
Cows	7.19	0.07	0.86 to 59.81
Goat (high contact)	4.85	0.16	0.54 to 44.03
Ate ice cream	**11.99**	**0.046**	**1.04 to 137.76**
Ate cotton candy	**51.90**	**0.008**	**2.77 to 970.67**

[a]OR, odds ratio; CI, confidence interval. Boldface type indicates variables with increased OR statistically significant at 95% level.

Discussion

This outbreak is the largest caused by VTEC O157 in visitors to an open farm and the first case-control study of risk factors for infection on an open farm in the United Kingdom. Our study has demonstrated a strong association with eating either ice cream or cotton candy and an increase in risk associated with goat and cow contact.

A case-control study among farm visitors in the United States in 2000 showed an association between *E. coli* O157 infection and contact with cattle, nail biting, and food purchase (12). Handwashing was protective in that study.

Considering sources of bias, particularly in the selection of controls or in gathering information, is important. Callers to a helpline are likely to differ in some ways from the other visitors, perhaps being better informed and more anxious. However, their ice cream or cotton candy eating habits are unlikely to differ. Information on known risk factors, such as handwashing and food history, may be susceptible to "rumination bias," that is, a tendency for those who have been ill to systematically bias the reporting of exposure. This bias would explain the apparent lack of protective effect of handwashing. Patients may have been more likely to recall eating ice cream, as this was one of the foods widely reported in the media as a possible source of infection in the early stages of the investigation. However, the association with cotton candy was unexpected, and there is no reason to think that patients were more likely to recall this than the controls.

The association between illness produced by VTEC O157 and contact with cows and goats reflects previous experience of direct transmission to humans (4,5,7). Cattle are regarded as the most important reservoir for VTEC O157 (13). However, the strong association with cotton candy and ice cream merits further discussion. Ice cream was supplied by the same local manufacturer to 65 other outlets in North Wales. Cotton candy was manufactured on site on the May bank holiday by a vendor using a process repeated at different fairs throughout North Wales. Illness associated with the ice cream or cotton candy was not reported elsewhere. However, both foods appeared to be strongly associated with the risk for illness. Both are particularly sticky, messy foods, and it is possible to envisage two mechanisms by which eating them makes the ingestion of *E. coli* O157 more likely. First, after eating one of these foods, sticky hands may be more prone to pick up contaminated organic matter from the environment or directly from animal coats by stroking. Secondly, to clean sticky hands, small children are likely to lick their fingers.

Our investigation reinforces existing advice (9,14) on handwashing, specifically that handwashing facilities should be positioned immediately adjacent to exit areas where animal contact is encouraged, and that one-way systems and adequate supervision can facilitate effective handwashing. Advice concerning the importance of super-

Table 3. Results of multivariable analysis of significant animal contact and food consumed for Bank Holiday attenders only and risk for illness (24 observations)[a]

	OR	p value	95% CI
Cows	13.65	0.14	0.42 to 438.71
Goat (high contact)	3.58	0.38	0.21 to 61.98
Ate ice cream	10.35	0.10	0.63 to 169.31
Ate cotton candy	38.44	0.07	0.71 to 2,092.60

[a]OR, odds ratio; CI, confidence interval.

vised handwashing before and after eating should be reinforced at the point of selling food. Our findings, and those of others (4,5,7,12,13) also suggest that calves may not be suitable animals for petting. In addition, we recommend specifically that food, particularly sweet and sticky food, only be sold and eaten in clean areas of the farm. Ideally, such sticky foods should be discouraged altogether.

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Pandemic Influenza and Healthcare Demand in the Netherlands: Scenario Analysis

Marianne L.L. van Genugten,* Marie-Louise A. Heijnen,* and Johannes C. Jager*

In accordance with World Health Organization guidelines, the Dutch Ministry of Health, Welfare and Sports designed a national plan to minimize effects of pandemic influenza. Within the scope of the Dutch pandemic preparedness plan, we were asked to estimate the magnitude of the problem in terms of the number of hospitalizations and deaths during an influenza pandemic. Using scenario analysis, we also examined the potential effects of intervention options. We describe and compare the scenarios developed to understand the potential impact of a pandemic (i.e., illness, hospitalizations, deaths), various interventions, and critical model parameters. Scenario analysis is a helpful tool for making policy decisions about the design and planning of outbreak control management on a national, regional, or local level.

In 1997, avian influenzavirus was shown to infect humans directly when an influenza virus A/H5N1 infected 18 people in Hong Kong; of those, six died (1,2). After this event, experts predicted that another influenza pandemic is highly likely, if not inevitable (3,4). The impact of a pandemic depends on factors such as the virulence of the pandemic virus and the availability of a vaccine. Because development is time-consuming, the vaccine would likely not be available in the early stages of a pandemic, and a major vaccine shortage would be expected (5). An influenza virus pandemic would likely cause substantial social disruption because of high rates of illness, sick leave, hospitalization, and death. Therefore, pandemic planning is essential to minimize influenza-related illness, death, and social disruption (5,6).

In accordance with World Health Organization guidelines, the Dutch Ministry of Health, Welfare and Sports developed a national plan to minimize or avert effects of pandemic influenza. Within the scope of the Dutch pandemic preparedness plan, we were asked to estimate the magnitude of the problem in terms of the expected number of hospitalizations and deaths during an influenza pandemic. We also estimated the potential effects of intervention options, including the use of the relatively new antiviral drugs, neuraminidase inhibitors (7,8).

One published study (9) has estimated the economic effects of an influenza pandemic. Meltzer et al. examined the possible effects of influenza vaccine-based interventions in terms of outpatient visits, hospitalizations, deaths, and related costs during a pandemic in the United States. More recently, different strategies for the control of interpandemic influenza for the elderly population in three European countries (England and Wales, France, and Germany) have been evaluated (10). Our objective was to examine the potential impact of pandemic influenza in the Netherlands and to analyze the effects of several (other than influenza vaccine–based) possible interventions in terms of hospitalizations and deaths.

Methods

Predicting when the next influenza pandemic will occur and how it will evolve is impossible, and the same is true for forecasting the number of persons who will become ill, be hospitalized, or die. Because of the many uncertainties, we performed a scenario analysis (11) that included consulting of experts and modeling. At a meeting of experts held to discuss an influenza pandemic in the Netherlands, specialists on influenza (virology, epidemiology, and surveillance) and on controlling epidemics and disasters gave their opinions about the formulated intervention scenarios, the assumptions made, and the value of critical parameters (12). A model was used to estimate the number of hospitalizations and deaths in the Netherlands for different scenarios. We also compared the number of expected hospitalizations and deaths for each of the different intervention scenarios to the number expected for the nonintervention scenario.

Scenarios

Various scenarios are possible, depending on whether influenza vaccine, pneumococcal vaccine, or antiviral drugs are available (among other factors). In all scenarios, we assumed a gross attack rate of 30%; we also assumed age-specific attack, hospitalization, and death rates and

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healthcare utilization (e.g., antibiotic drug prescription) as in a regular epidemic. Table 1 shows the base-case assumptions in the various scenarios. Following are descriptions of the scenarios considered relevant and sufficiently realistic by the specialists who participated in the meeting of experts.

Nonintervention Scenario

The nonintervention scenario is a "worst case" situation in which no intervention is possible. The scenario includes a pandemic influenza for which no vaccine is available and only regular care and regularly prescribed antibiotic drugs are provided. In the base case, we assume a gross attack rate of 30%; an age-specific attack; and hospitalization, death rates, and healthcare utilization as in a regular epidemic.

Influenza Vaccination Scenario

In this scenario, when an influenza vaccine becomes available, two possible strategies are considered: 1) vaccination of risk groups including persons ≥65 years of age ($n = 2.78\times10^6$) and healthcare workers ($n = 0.80\times10^6$) and 2) vaccination of the total population ($n = 15.6\times10^6$). In the base case, influenza vaccination is assumed to be 56% effective in preventing hospitalizations and deaths in persons ≥65 years of age (15), and 80% effective in those ≤64 years of age (Table 1) (13,14).

Pneumococcal Vaccination Scenario

In the absence of a vaccine available at the beginning of a pandemic, the Dutch Health Council recommends providing influenza risk groups (including those ≥65 years of age; $n = 2.78\times10^6$) with pneumococcal vaccination (18), which is a 23-valent vaccine assumed to prevent invasive infections caused by *Streptococcus pneumoniae*, one of the possible complications of influenza. For the base case, we assumed that 50% of hospitalizations and deaths from influenza-related pneumonia are caused by invasive pneumococcal infection and that pneumococcal vaccination prevents 80% of invasive infections caused by vaccine serotypes (Table 1) (16,17). In the Netherlands, 80% of serotypes involved in invasive pneumococcal infections are covered by the 23-valent vaccine, which results in a vaccine effectiveness of 64% against invasive pneumococcal infections.

Therapeutic Use of Neuraminidase Inhibitors Scenario

This scenario includes the use of neuraminidase inhibitors. When taken within 48 hours after onset of symptoms and continued for 5 days, neuraminidase inhibitors (zanamivir and oseltamivir) (19) reduce the duration and seriousness of influenza by 1 to 2 days for adults (20–24), children (22,25,26), and persons at high risk (22,27–29). However, the effectiveness of neuraminidase inhibitors for preventing hospitalizations and deaths (our outcome parameters) is unknown. Therefore, we assumed that 25% to 75% of the hospitalizations and deaths attributed to influenza would be avoided by therapeutic use of neuraminidase inhibitors (12) in this scenario (each person with an influenzalike illness begins the medication within 48 hours after the first symptoms). An advantage of therapeutic use of neuraminidase inhibitors is that antibodies are formed (26) because infection is not prevented; thus protection against an infection resulting from the same virus is built up, as in an untreated infection.

Table 1. Assumptions made for influenza pandemic scenario analysis, the Netherlands

Scenario	Assumptions in base case	Assumptions in sensitivity analysis
No intervention	Gross attack rate of 30%; age-specific attack, hospitalization, and death rates as in regular epidemic; and healthcare utilization as in regular epidemic.	Gross attack rate of 10% and 50%; age-specific attack rates (see Table 4); and complication rates for a) persons ≤64 y of age x 2 and b) persons at low risk equal to persons at high risk.
Influenza vaccination of risk groups (including persons ≥65 y of age) and healthcare workers	Gross attack rate of 30%; age-specific attack, hospitalization, and death rates as in regular epidemic; and vaccine efficacy 80% (≤64 y of age) (13,14) and 56% (≥65 y) (15) to prevent hospitalizations and deaths	Gross attack rate of 10% and 50%; age-specific attack rates (see Table 4); complication rates for a) age group ≤64 y times 2 and b) persons at low risk equal to persons at high risk; influenza vaccine efficacy a) 80% for all ages and b) 40% for age group ≤64[a] and 30% for age group ≥65[b].
Pneumococcal vaccination of influenza of risk groups (including persons aged ≥65 y)	Gross attack rate of 30%; age-specific attack, hospitalization, and death rates as in regular epidemic; 50% pneumococcal-related hospitalizations; and vaccine efficacy 64% against invasive infections (16,17).	Gross attack rate of 10% and 50%; age-specific attack rates (see Table 4); complication rates for a) persons ≤64 y of age x 2 and b) persons at low risk equal to persons at high risk; 25% and 75% pneumococcal-related hospitalizations; and vaccine efficacy 25% and 75%.
Therapeutic use of neuraminidase inhibitors for all patients with influenzalike illness	Gross attack rate of 30%; age-specific attack, hospitalization, and death rates as in regular epidemic; and 50% reduction of hospitalizations and deaths.	Gross attack rate of 10% and 50%; age-specific attack rates (see Table 4); complication rates for a) persons ≤64 y of age times 2 and b) persons at low risk equal to persons at high risk.; and 25% to 75% reduction of hospitalizations and deaths.

[a]Minimum variant based (9).
[b]Maximum variant assumes 80% efficacy for all ages.

Although neuraminidase inhibitors have proven to be effective prophylactically (27,30–32), the specialists were unanimous in their opinion that using neuraminidase inhibitors prophylactically on a large scale in a pandemic is not feasible because they need to be taken as long as the threat of influenza virus infection lasts. The medication would therefore need to be taken for at least several weeks to several months in a pandemic. An enormous stockpile of neuraminidase inhibitors would be required for the Dutch population; compliance, in the course of time, would likely diminish. In this scenario, using this medication for prophylactic purposes might merely postpone the pandemic, and the disease might emerge at the moment that most of the population stops the prophylaxis unless an effective and safe vaccine is available in sufficient amount at that time.

The specialists considered neuraminidase inhibitors to be more suitable than previous antiviral medicines (amantadine and rimantadine), which lead to viral resistance, have serious side effects, and are only effective against influenza A (7,8,14). Neuraminidase inhibitors are effective against influenza A and B and have not generated much resistance thus far (19,33,34); they appear to be safe and have seldom caused serious side effects (34–36).

Model and Data

Building a mathematical model of influenza spread is difficult because of yearly differences in virus transmission and virulence, lack of understanding of the factors affecting the spread of influenza, and shortage of population-based data (9,37). We used a static model (12) that estimates the numbers of hospitalizations and deaths in the Netherlands by using data from earlier influenza epidemics and literature review. The model was implemented by using an Excel spreadsheet (Microsoft Corp., Redmond, CA) (Figure 1). In the model, we distinguished three age groups (≤19 years, 20–64 years, and ≥65 years) by low or high risk (susceptibility to the complications of hospitalization and death) for influenza. The population not protected against influenza depends on vaccination coverage and vaccine and neuraminidase efficacy; all can be different in each scenario. We calculated the number of influenza cases in each age group at low or high risk for influenza by multiplying numbers not protected against influenza and attack rates. We calculated the absolute number of hospitalizations and deaths in each age group at low or high risk for influenza by multiplying the calculated number of influenza cases and the influenza-specific complication (hospitalization or death) rates. The case-specific complication rates in each age group at low or high risk for influenza are computed from general population–specific complication rates, current vaccination degree, and vaccine efficacy by assuming that during a regular epidemic

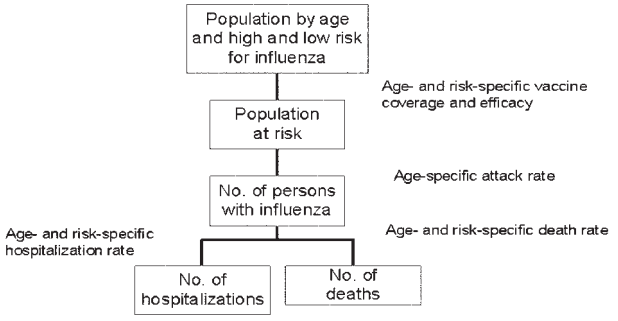

Figure 1. Schematic view of calculation model used for scenario analysis.

10% of the population becomes ill (12). The age distribution of the influenza cases in the general population is assumed to be equal to the age distribution of persons consulting their general practitioner for influenzalike illness. Table 2 shows the values of the basic input variables.

Sensitivity Analyses

Sensitivity analyses were performed on the gross attack rate, age-specific attack, hospitalization and death rates, and on efficacy of vaccines and neuraminidase inhibitors. Table 1 describes assumptions used in sensitivity analysis.

Results

Results are shown in terms of number of hospitalizations and deaths (prevented) in relation to doses of vaccines or antiviral drugs needed. During a regular influenza epidemic in the Netherlands, approximately 1,900 hospitalizations and 800 deaths related to influenza occur. The nonintervention scenario of an influenza pandemic with a gross attack rate of 30% and no interventions available could lead to as many as 10,000 influenza-related hospitalizations and >4,000 deaths (Figures 2 and 3).

The influenza vaccination scenario could prevent >6,000 (>60%) of hospitalizations and >2,200 (>55%) of deaths. Vaccination of the total population requires 15.6 million doses of vaccine; vaccination only of risk groups for influenza (including persons ≥65 years of age and healthcare workers) requires 3.6 million vaccines. The pneumoccoccal vaccination scenario, which requires 2.8 million doses of vaccine, could prevent 2,600 (25%) of the hospitalizations and 140 (3.5%) of the deaths. The therapeutic use of neuraminidase inhibitors scenario could prevent 5,000 hospitalizations and 2,000 deaths (assuming 50% efficacy) and would require 4.7 million prescriptions of neuraminidase inhibitors.

A decrease (increase) in the gross attack rate to 10% (to 50%) shows a similar decrease (increase) in the absolute number of expected hospitalizations and deaths. Assuming different gross attack rates does not change the percentage of hospitalizations and deaths that might be avoided in the

Table 2. Input variables used to calculate potential impact of influenza pandemic in terms of healthcare outcomes and the effect of various interventions, the Netherlands

Input variable	Age groups (y) ≤19	20–64	≥65	Sources
Population	3.8×10^6	9.7×10^6	2.1×10^6	Statistics Netherlands
Population at high risk	0.09×10^6	0.6×10^6	0.7×10^6	(38–40)
Age distribution of influenza cases	34.3	60.4	5.2	As in a regular epidemic in general practice (41)[a]
Current vaccination degree				(42,43)
Population at low risk	0.02	0.05	0.20	
Population at high risk	0.65	0.75	0.80	
Efficacy influenza vaccine	80%	80%	80%	(13–15)
Invasive pneumococcal infections				(12,16,17)
Related hospitalizations	50%	50%	50%	
Efficacy vaccine	64%	64%	64%	
Hospitalization rate (per 100,000) for influenza				As in a regular epidemic (44)[a]
Population at low risk	0.1	0.1	2	
Population at high risk	28	28	10	
Hospitalization rate (per 100,000) for influenza-related pneumonia				As in a regular epidemic (44)[a]
Population at low risk	0.3	0.3	38	
Population at high risk	72	72	175	
Death rate (per 100,000)				As in a regular epidemic (45)[a]
Low risk population	0.6	0.6	26.2	
High risk population	29.6	29.6	84.9	

[a]Assuming that during a regular epidemic 10% of the population becomes ill.

different scenarios (Table 3). By using a range of age-specific attack rates (Table 4) for the nonintervention scenario, we estimated that the number of hospitalizations ranged from 7,500 to >19,000 and the number of deaths from 2,700 to approximately 9,000 (Table 5). The variation in the number of hospitalizations and deaths in each of the scenarios is substantial. However, assuming different age-specific attack rates leads to little difference in the percentage of hospitalizations and deaths that might be avoided by a certain intervention.

If one assumes that complication (i.e., hospitalization and death) rates for low-risk persons are equal to the complication rates for high-risk persons, the number of hospitalizations and deaths increases dramatically. In the nonintervention scenario, we estimated >64,000 hospitalizations (>10,000 in the base case) and approximately 10,000 deaths (approximately 4,000 in the base case). The number of avoided hospitalizations ranges from almost 6,000 in the pneumococcal vaccination scenario to >45,000 in the influenza vaccination (of the total population) scenario, and the number of avoided deaths ranges from 1,000 to >6,000 (Table 6). In the scenario with influenza vaccination of risk groups, this assumption leads to a decrease in the percentage of hospitalizations and deaths that might be avoided, 21% (base case 61%) and 47% (base case 56%), respectively. In the scenario with pneumococcal vaccination of risk groups, the percentage of hospitalizations and deaths that might be avoided decreases to 9% (base case 31%) and 1% (base case 3%), respectively.

Low and high levels for age-specific influenza vaccine efficacy show that the number of expected hospitalizations varies from almost 2,000 to >6,900 and the number of deaths varies from almost 800 to >2,800 (Table 7). These numbers are equal to a range of 30% to 80% in the percentage of the number of hospitalizations and deaths that might be avoided (base case 55% to 60%).

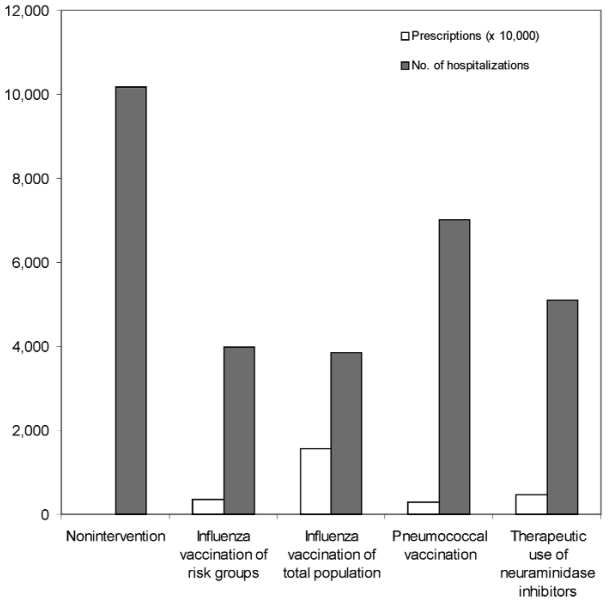

Figure 2. Number of hospitalizations and required prescriptions in the various scenarios.

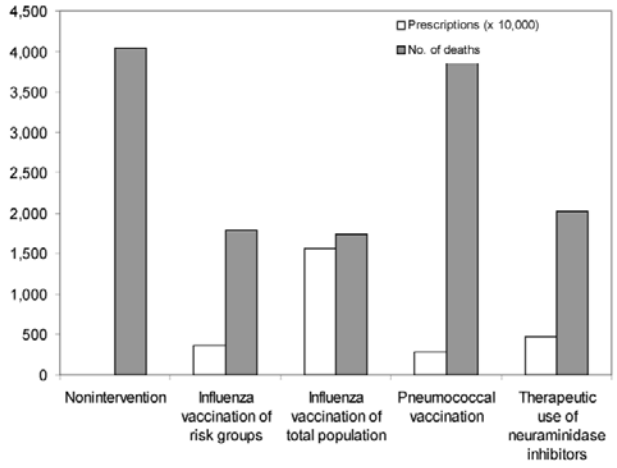

Figure 3. Number of deaths and required prescriptions in the various scenarios.

For the pneumococcal vaccine scenario, we tested two parameters: the percentage of complications (25% to 75%) to be prevented by pneumococcal vaccination and the pneumococcal vaccine efficacy (also 25% to 75%). Our results showed that the number of expected hospitalizations varies from 5,400 to 8,950, the number of deaths varies from >3,800 to 4,000 (Table 8). These values are equal to a range of 12% to 47% (base case 31%) and 1% to 5% (base case 3%) in the percentage of the number of hospitalizations and deaths that might be avoided. When assuming 25% to 75% effectiveness for the neuraminidase inhibitors scenario, we also estimated that between 25% and 75% of the number of hospitalizations and deaths can be avoided.

Discussion

The nonintervention scenario describes a pandemic situation in which no interventions are available; such an influenza pandemic, with a gross attack rate of 30%, would result in five times as many influenza-related hospitalizations and deaths as in a regular influenza epidemic with the current degree of vaccination, mostly in persons ≥65 years of age. Sensitivity analysis shows that varying the gross attack rate does not change the percentage of hospitalizations and deaths that might be avoided in the different scenarios. Varying the age-specific attack, hospitalization, and death rates has a large impact on the estimated number of hospitalizations and deaths. However, the impact is less in terms of the percentage of the number of hospitalizations and deaths that might be avoided by the various interventions.

Influenza vaccination may prevent many hospitalizations and deaths. The influenza vaccination scenario suggests that when assuming the age-specific complication rates of a regular epidemic, vaccination of the total population compared to vaccination of healthcare workers and the groups at risk for influenza would do little to avert hospitalizations and deaths. However, sensitivity analysis shows this result to be quite sensitive to the assumptions of the complication rates by age. As a consequence of higher complication rates in lower age and risk groups, the percentage of averted hospitalizations and deaths substantially decreases in the scenario's pneumococcal and influenza vaccination of risk groups for influenza.

Only a pandemic itself can provide better estimates of the age-specific attack and complication rates, but these analyses show a range of what might be expected. While the likelihood of an available influenza vaccine in the

Table 3. Hospitalizations and deaths in the scenario analysis of influenza pandemic[a]

	No. of hospitalizations			No. of deaths		
Scenario	Base case	Gross attack rate 10%	Gross attack rate 50%	Base case	Gross attack rate 10%	Gross attack rate 50%
Nonintervention	10,186	3,395	16,977	4,040	1,347	6,733
Influenza vaccination						
Total population	3,847	1,282	6,412	1,738	579	2,896
Risk groups	3,968	1,223	6,614	1,789	596	2,981
Pneumococcal vaccination	7,008	2,326	11,679	3,903	1,301	6,505
Neuraminidase inhibitors	5,093	1,698	8,489	2,020	673	3,367

[a]Assuming gross attack rates of 10% and 50%.

Table 4. Alternative age-specific attack rates in scenario analysis for pandemic influenza, the Netherlands[a]

			Age groups affected in proportion of			
Age (y)	Age groups affected as in regular epidemic	Age groups equally affected	1:1:2	1:2:1	2:1:1	Previous pandemics[b]
≤19	37.4	30.0	26.4	18.5	48.3	49.3
20–64	28.6	30.0	26.4	37.0	24.1	25.6
≥65	23.1	30.0	52.9	18.5	24.1	15.0

[a]Gross attack rate 30%.

[b]Distribution from Meltzer et al. (9) based on previous pandemics.

Table 5. Hospitalizations and deaths in various scenarios for alternative age-specific attack rates

	No. of hospitalizations per age group						No. of deaths per age group					
				Age group proportion						Age group proportion		
Scenario	Regular epidemic	Groups equally affected	1:1:2	1:2:1	2:1:1	Previous pandemics[b]	Regular epidemic	Groups equally affected	1:1:2	1:2:1	2:1:1	Previous pandemics[b]
Nonintervention	10,186	12,478	19,630	9,184	10,252	7,541	4.040	5,199	9,009	3,288	4,197	2,746
Influenza vaccination												
Total population	3,847	4,844	8,068	3,285	3,939	2,716	1,738	2,245	3,929	1,401	1,809	1,169
Risk groups	3,968	4,962	8,171	3,410	4,058	2,840	1,789	2,294	3,972	1,454	1,860	1,222
Pneumococcal vaccination	7,008	8,574	13,460	6,323	7,053	5,200	3,903	5,015	8,697	3,178	4,054	2,654
Neuraminidase inhibitors	5,093	6,239	9,815	4,592	5,126	3,771	2,020	2,600	4,505	1,644	2,099	1,373

[a]Gross attack rate 30%.
[b]Distribution from Meltzer et al. (9) based on previous pandemics.

beginning of a pandemic is low, the next best option seems to be the therapeutic use of neuraminidase inhibitors. However, this option has three major considerations: 1) effective use of neuraminidase inhibitors depends greatly on the assumption of 50% effectiveness to prevent hospitalizations and deaths; 2) every patient with influenzalike illness must begin medication within 48 hours after onset of symptoms (a logistically complicated task); and 3) a sufficient stock of neuraminidase inhibitors must be available, which is currently not the case. In our current approach, we probably underestimated the effect of influenza vaccination and the therapeutic use of neuraminidase inhibitors because we did not take into account the specific features of influenza as an infectious transmissible disease.

Pneumococcal vaccination could prevent 31% of the hospitalizations and 3.4% of the deaths. This intervention is the least effective because pneumococcal vaccination prevents only one complication of influenza (i.e., invasive pneumococcal infections). In contrast to hospitalizations, few deaths might be prevented by pneumococcal vaccination because relatively more excess hospitalizations than deaths are attributable to influenza-related pneumonia. An advantage of this intervention is that pneumococcal vaccination can be done before the pandemic starts since the vaccine is effective in preventing invasive pneumococcal infections for approximately 5 years (15). As expected, sensitivity analysis showed that lower vaccine effectiveness results in less hospitalizations and deaths prevented. In the next pandemic, if pneumoccocal infections occur more often as a complication of influenza than in the base case, using this intervention would prevent increased hospitalizations and deaths.

The objective of our study was to examine the potential impact (in terms of hospitalizations and deaths) of pandemic influenza in the Netherlands and to analyze the

Table 6. Hospitalizations and deaths in various scenarios for alternative complication rates[a]

	No. of hospitalizations			No. of deaths		
		Hospitalization and death rate			Hospitalization and death rate	
Scenario	Base case	Age group ≤64 y x 2	Low risk to high risk rate	Base case	Age group ≤64 y x 2	Low risk to high risk rate
Nonintervention	10,186	12,830	64,425	4,040	4,207	10,087
Influenza vaccination						
Total population	3,847	4,376	16,798	1,738	1,771	3,981
Risk groups	3,968	4,617	50,935	1,789	1,873	5,333
Pneumococcal vaccination	7,008	8,857	58,597	3,903	4,066	9,950
Neuraminidase inhibitors	5,093	6,415	32,212	2,020	2,104	5,043

[a]See Table 1.

Table 7. Hospitalizations and deaths for alternative influenza vaccine efficacy

	No. of hospitalizations			No. of deaths		
		Vaccine efficacy			Vaccine efficacy	
Scenario	Base case	All age groups equal to 80%[b]	Age groups ≤64 y = 40%; for ≥65 = 30%[c]	Base case	All age groups equal to 80%	Age groups ≤64 y = 40%; for ≥65 y = 30%[c]
Nonintervention	10,186	10,186	10,186	4,040	4,040	4,040
Influenza vaccination						
Total population	3,847	2,037	6,866	1,738	808	2,811
Risk groups	3,968	2,158	6,926	1,789	859	2,837

[a]See Table 1.
[b]Minimum variant based on Meltzer et al. (9).
[c]Maximum variant assumes 80% efficacy for all ages.

Table 8. Hospitalizations and deaths for alternative values for pneumococcal related variables

	No. of hospitalizations					No. of deaths				
		Reduction of complications		Vaccine efficacy			Reduction of complications		Vaccine efficacy	
Scenario	Base case	25%	75%	25%	75%	Base case	25%	75%	25%	75%
Non intervention	10,186	10,186	10,186	10,186	10,186	4,040	4,040	4,040	4,040	4,040
Pneumococcal vaccination	7,008	8,597	5,418	8,945	7,703	3,903	3,971	3,834	3,986	3,933

[a]See Table 1

effects of several possible interventions. Ideally, after a pandemic has started, the influenza vaccine should be available and administered as quickly as possible following a prioritized scheme. In the Netherlands, developing this scheme is a governmental task. The scheme may be dependent on the actual (observed) age-specific attack and complication rates. However, at the start of the pandemic, no vaccine is expected to be available. Based on our analysis and assumptions, we conclude that a combined strategy of pneumococcal vaccination of risk groups for influenza together with the therapeutic use of neuraminidase inhibitors for all patients with influenzalike illness (within 48 hours after onset of symptoms) is the best strategy in preventing hospitalizations and deaths.

This recommendation is not valid if therapeutic use of neuraminidase inhibitors is shown to be ineffective in preventing influenza-related hospitalizations and deaths. Also, if the next pandemic shows that invasive pneumococcal infections are not a complication of influenza, pneumococcal vaccination is no longer a valid intervention. Because these questions are still unanswered, we also recommend ongoing research in the field of vaccine production techniques.

To prepare effectively for the next pandemic, the Dutch government will continue to investigate stockpiling neuraminidase inhibitors and securing influenza vaccine supply during a pandemic.

Our scenario analysis provides information about reducing the effects of a pandemic to a minimum, both regionally and nationally, to those who must prepare for the control of an actual pandemic. The insights from the scenario analysis provide a possible order of magnitude for providing healthcare (regional data were also calculated; data not shown). Furthermore, by using a model and a set of assumptions, we compared the effects of various interventions on the demand for care. Scenario analysis provided insight into which parameters have the most influence on the outcome variables (the age-specific attack and complication rates). If outbreaks of a new, potentially pandemic, influenza virus occur abroad and if these outbreaks yield real information about the attack and complication rates by age group, we can use these values in our model to update the estimate of the demand for care that can be expected in the Netherlands, nationally and regionally. Other countries might also use a similar approach to support their pandemic preparedness planning.

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Estimating the Incidence of Typhoid Fever and Other Febrile Illnesses in Developing Countries

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To measure the incidence of typhoid fever and other febrile illnesses in Bilbeis District, Egypt, we conducted a household survey to determine patterns of health seeking among persons with fever. Then we established surveillance for 4 months among a representative sample of health providers who saw febrile patients. Health providers collected epidemiologic information and blood (for culture and serologic testing) from eligible patients. After adjusting for the provider sampling scheme, test sensitivity, and seasonality, we estimated that the incidence of typhoid fever was 13/100,000 persons per year, and the incidence of brucellosis was 18/100,000 persons per year in the district. This surveillance tool could have wide applications for surveillance for febrile illness in developing countries.

Measuring the incidence of febrile illness caused by various pathogens poses a major public health challenge because hospital-based approaches capture only a fraction of patients, clinical diagnosis is usually unreliable, and diagnostic tests are often not available in disease-endemic countries (1). Consequently, the incidence and relative importance of the etiologic agents of the febrile illness remain unknown in many parts of the world. Public health personnel have insufficient data to make the disease burden (incidence, illness, and death) estimates to guide priorities for the use of scarce health resources (2) and to help refine policy on the empiric management of febrile patients (3).

Attempts to measure the incidence of febrile illness have been hampered by problems associated with surveillance sensitivity and surveillance specificity. Sensitivity is determined largely by the placement of the surveillance system within the healthcare system and the completeness of enrollment of case-patients (Figure). Although conducting surveillance at the tertiary hospital level is attractive from the perspective of laboratory capacity and infrastructure, such surveillance captures only the most severe illnesses in persons who have access to hospital care. Hospital-based approaches tend to underestimate disease incidence. Routine door-to-door visits to every household in a community to identify febrile persons and then collect diagnostic specimens is highly sensitive but limited by cost and time considerations (4). Specificity is determined largely by the diagnostic criteria used in the surveillance case definition. Syndrome-based surveillance requires no laboratory capacity, but lacks specificity because the causes of febrile illnesses may be clinically indistinguishable. Therefore, syndrome-based surveillance frequently results in classification errors. To maximize specificity, the case definition for the febrile illnesses under surveillance must include a positive result from a reliable diagnostic test.

A sensitive and specific surveillance system that accurately measures the incidence and causes of febrile illness in a country or region must be able to detect cases as close as possible to the population level (Figure) and must be supported by modern laboratory diagnostic capacity. Because such surveillance is labor-intensive and expensive, a rapid method that measures incidence and cause in sentinel populations of a country or region over a finite period of time is needed. Such sentinel surveillance could be repeated at intervals to detect changing patterns of disease.

We developed a rapid sentinel surveillance tool to determine the causes and measure the incidence of febrile illness. We pilot-tested this tool in Egypt, where the Egypt Ministry of Health and Population and the U.S. Naval

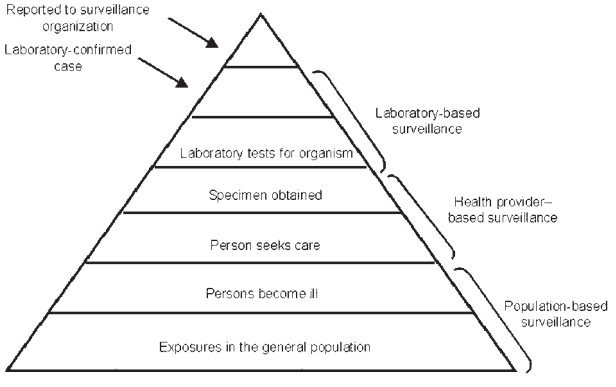

Figure. Febrile illness surveillance pyramid.

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Medical Research Unit No. 3 (NAMRU-3) have recently collaborated to expand laboratory capacity at district fever hospitals as part of a plan to strengthen national hospital-based surveillance for febrile illnesses. Fever hospitals are tertiary referral centers for persons with suspected infectious disease. By using our surveillance tool in concert with Egypt's expanded laboratory capacity, we aimed to determine the etiologic agent and to measure the incidence of the leading causes of prolonged fever in Bilbeis District.

Methods

Study Site

Bilbeis District, Sharkia Governorate, Lower Egypt, was chosen as the study site. Bilbeis District has a population of 664,000 and comprises a rural hinterland and centrally located Bilbeis City. Rural Bilbeis District comprises scattered villages and hamlets that rely largely on subsistence agriculture. Bilbeis City consists of high-density single- and multiple-story dwellings. The relatively close proximity of this district to Cairo provided practical advantages for epidemiologic and laboratory support.

Household Survey

We conducted a household survey during August 2000 and January 2001 in Bilbeis and neighboring Fakkous Districts, Lower Egypt. The survey was part of a larger study that evaluated injection practices in several parts of Egypt (5). Our goal was to determine patterns of health-seeking behavior among persons reporting prolonged fever (self-diagnosed fever ≥3 days' duration) in Bilbeis and Fakkous Districts during the 3-month period before the interview. The two districts were divided into 40 rural sites of approximately equal population. Four of the sites were randomly selected. In these four rural sites, a census was conducted of all households and household members. The study team spent 1 week working in each rural site. All persons living in each household were invited to participate in the interview by answering a structured questionnaire. If a household member was absent on the day of the visit, the study team returned at least once during the 1-week period. For children <10 years of age, the head of the household was interviewed.

Sentinel Surveillance

We obtained a contemporary census of all district health services and health providers from the Bilbeis District Health Office and used the findings of the household survey to identify categories of health providers who were seeing patients with prolonged fever. We used data from the census of district health services and health providers as the sampling frame from which to select health providers for the febrile illness surveillance system. One district fever hospital, 11 fever specialists, and 68 primary care providers (general practitioners, internal medicine physicians, pediatricians, and rural health unit doctors) were recorded in the contemporary health services and health provider census of the Bilbeis District Health Office. We conducted the surveillance from July through October 2001 at the fever hospital, among the 11 district fever specialists, and among a random selection of 10 (15%) of 68 other representative health providers.

During the 4-month study period, all persons of ages ≥6 months who visited a surveillance health provider in Bilbeis District with current fever of ≥3 days' duration were invited to participate. After obtaining informed consent from these febrile patients, health providers administered a brief questionnaire that captured demographic and clinical information; blood was collected for culture and serologic testing. Health providers were given a small financial incentive to compensate for the additional time required to enroll patients. Persons <6 months of age were not included because this group is understood to be at low risk for typhoid fever, in part because of predominant or exclusive breastfeeding (4). Ethical approval was obtained from the Institutional Review Boards of NAMRU-3 and the Centers for Disease Control and Prevention.

Laboratory Capacity and Methods

We trained healthcare providers on obtaining blood culture, sterile technique, and needle safety and supplied these providers with materials for venipuncture and blood culture. Couriers visited each provider every day to ensure adequate laboratory supplies and transport laboratory samples and test results. Microbiology laboratory technicians at the Bilbeis fever hospital were trained in blood culture technology and retrained in basic bacteriology. The medical microbiology laboratory was equipped with biological safety equipment, an incubator, and other materials necessary to process blood cultures and identify bacteria. The NAMRU-3 bacteriology laboratory in Cairo provided training, quality control on all samples and bacterial isolates, a reference laboratory, and special test capacity.

The Phase2 bi-phasic blood culture system (PML Microbiologicals, Wilsonville, OR) was used. We incubated bottles for 14 days at 35°C and observed them daily for signs of microbial growth. Growth in broth or on agar paddles was examined by Gram stain and was subcultured to solid media for identification. Serologic testing for *Brucella* spp. was performed by standard tube agglutination with *Brucella abortus* antigen (SA Scientific, San Antonio, TX).

Incidence Calculations

Incidence was calculated for each examined disease after accounting for the provider-sampling scheme, test

sensitivity and specificity, and seasonality. Multipliers to account for the provider-sampling scheme were derived arithmetically by using the provider populations sampled in our study as the numerator and the provider populations known from the contemporary census of all district health services and health providers as the denominator. Because the sentinel surveillance system included the only district fever hospital and all district fever specialists, no multiplier was applied to cases detected at these sites. However, to account for sampling, only 15% of primary health providers in the district, a multiplier of 6.8 was applied to cases detected among primary healthcare providers.

Multipliers to account for shortcomings of test sensitivity were derived by reviewing published systematic studies of the performance of the diagnosed tests used in our study compared with standard criterion tests. The sensitivity of a single blood culture for the diagnosis of typhoid fever has been estimated as 50% when compared with bone marrow aspirate culture (6); therefore, a multiplier of 2.0 was applied to account for blood culture–negative typhoid fever. Because most brucellosis cases can be detected with the combination of blood culture and the tube agglutination assay, no multiplier was applied to account for test sensitivity for brucellosis. For the purposes of incidence calculations, test specificity for typhoid fever and brucellosis was assumed to approach 100%.

Multipliers to account for seasonal variation in disease incidence were derived from syndrome- and laboratory-based febrile illness surveillance systems. Because approximately 45% of typhoid fever occurs from June through October in Egypt (national syndrome-based surveillance system for typhoid fever, unpub. 2000), a multiplier of 2.2 was applied to adjust for the whole year. A similar seasonal pattern occurs for brucellosis (national acute febrile illness surveillance system, unpub. data, 2000) (Table).

Statistical Methods

Data were stored and analyzed with Epi Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA). Incidence calculations were made by using Microsoft Excel 2000 (Microsoft Corp. Redmond, WA) spreadsheets.

Results

Household Survey

A census of the four randomly selected rural sites recorded 369 households. Interviews were completed in all 369 randomly selected households. Of the 2,421 persons eligible for interview in survey households, 363 (15.0%) could not be interviewed because the participant was absent during the 1-week survey period. No eligible person refused to be interviewed. In total, 2,058 (85.0%) of 2,421 eligible persons, or their guardians, were interviewed. Of persons interviewed, 474 (23.0%) reported having fever of ≥3 days' duration (i.e., prolonged fever) during the previous 3 months. Of those reporting prolonged fever, 379 (80.0%) sought care from a health provider. Of those seeking care from a health provider, 340 (89.7%) saw a physician, 32 (8.4%) saw a pharmacist, and 7 (1.8%) saw a layperson. Of the 340 who saw physicians, 274 (80.6%) saw a private physician, 36 (10.6%) saw a rural health unit physician, 19 (5.6%) saw a physician at a district general hospital, 7 (2.1%) saw a physician working for a health insurance organization, and 1 (0.3%) saw a physician at the district fever hospital.

Sentinel Surveillance

In total, 449 patients with prolonged fever were enrolled at the sentinel surveillance sites. No eligible patients refused to participate. *Salmonella enterica* serotype Typhi (*Salmonella* Typhi) was isolated by blood culture from 19 (4.2%) patients. The median age of patients with typhoid fever was 22 years (range 5–60 years), and 5 (26.3%) patients were female. *Brucella* spp. were isolated by blood culture from 15 (3.3%) patients, and brucellosis was confirmed by positive tube agglutination assay (titer of ≥1:160) for another 16 (3.6%). The median age of patients with brucellosis was 31 years (range 11–60 years); 12 (38.7%) patients were female. *Escherichia coli* and *Hemophilus influenzae* serotype b were each isolated by blood culture from one patient. No non-Typhi *Salmonella* serotypes were isolated.

The contamination of blood cultures with skin flora (e.g., coagulase-negative *Staphylococcus*, diphtheroids), resulting from poor sterile technique, was a problem dur-

Table. Incidence estimates for typhoid fever and brucellosis, Bilbeis District, Egypt, 2001

	No. of cases captured by surveillance site type Crude (adjusted[a])				Test sensitivity multiplier	Seasonality multiplier	Total cases	Incidence (/100,000)
Disease	Fever hospital	Fever specialist	Primary provider	Total				
Typhoid fever	6.0 (6.0)	13.0 (13.0)	0.0	19.0 (19.0)	2.0	2.2	83.6	12.6
Brucellosis	15.0 (15.0)	12.0 (12.0)	4.0 (27.2)	31.0 (54.2)	1.0	2.2	119.2	18.0

[a]Adjusted for health provider sampling scheme. No multiplier is applied for cases identified at the fever hospital and among fever specialists. A multiplier of 6.8 is applied for cases identified among primary providers.

ing the early part of the study. Active monitoring and intensive retraining of participating health providers reduced the blood culture contamination rate from 15% during the first 2 months of the study to 7% during the second 2 months of the study ($p<0.01$).

Of patients with brucellosis, 26 (87.1%) of 31 were diagnosed with and treated clinically for typhoid fever. In total, 302 (71%) of 423 patients were already using an antimicrobial agent at the time they sought treatment by a health provider participating in the sentinel surveillance system. Patients most frequently reported taking amoxicillin and chloramphenicol. However, no significant association was found between current antimicrobial therapy and yield of pathogens by blood culture.

Incidence Calculations

After we made adjustments to account for the provider-sampling scheme, test sensitivity, and seasonality, we estimated the annual typhoid incidence rate as 13/100,000 persons and the annual brucellosis incidence rate as 18/100,000 persons. The multipliers and calculations used to derive these estimates are summarized in the Table.

Discussion

Before our study, the most reliable existing estimates of the typhoid fever incidence rates in Egypt were established during typhoid vaccine studies conducted more than two decades earlier. These studies documented an annual typhoid fever incidence of 209/100,000 persons in 1972–1973 (7) and of 48/100,000 persons in 1978–1981 (8,9) among school-aged children in Alexandria, Egypt. Vaccine studies may overestimate typhoid fever incidence because they may be preferentially conducted in areas of known high incidence of typhoid and in groups at high risk of acquiring typhoid fever (e.g., school-aged children). Our study, conducted among all age groups in a single district, showed annual typhoid fever incidence rates that were lower, at 13/100,000 persons. This finding is consistent with a study design that was not targeted to a high incidence population. A lower typhoid fever incidence may also be consistent with reductions of other enteric diseases reported in Egypt, resulting from improved management of diarrheal disease (10) and the large and growing proportion of persons living in both rural and urban areas who have access to safe water (11).

Our study demonstrated that brucellosis was as important as typhoid fever as a cause of prolonged fever in Bilbeis District. Brucellosis has increasingly become recognized as a public health problem in Egypt, as it has in Kuwait and other countries in the Middle East. Our estimated annual rate of 18/100,000 persons approaches that found in Kuwait during the 1980s (12). Because brucellosis and typhoid fever have similar signs and symptoms, brucellosis frequently was misdiagnosed as typhoid fever, resulting in provision of inadequate antimicrobial therapy.

Healthcare provider–based surveillance previously has been used to capture typhoid fever cases for vaccine studies (13) and to measure typhoid fever incidence (14). However, such an approach requires that febrile persons report to the health providers participating in the study (Figure). We conducted a household survey to assess patterns of health-seeking behavior in Bilbeis District before implementing surveillance. We improved the efficiency of this step by integrating questions of health-seeking behavior for febrile persons with an existing population-based survey (5). The reliability of the household survey data could be improved by following classic cluster sampling methods (15,16). One way to achieve this would be integration with national demographic and health surveys that use cluster-sampling methods and are conducted at regular intervals in many developing countries.

Identifying, assessing, and strengthening a central laboratory capacity is a key foundational step for implementing our surveillance tool. Our sentinel surveillance study was built on recently expanded laboratory capacity within Egypt's district fever hospitals. Others investigators have successfully identified, assessed, and strengthened central laboratory capacity for sentinel hospital-based studies of febrile illness (17–19). We extended capacity beyond the tertiary hospital and into the community to determine the etiologic agents and to estimate the incidence of febrile illness closer to the population level.

Several factors must be considered when assessing the accuracy of this surveillance tool for measuring the incidence of febrile illnesses. The febrile illness surveillance tool may not capture mild disease. Although mild illness does not contribute substantially to disease burden, using a broader case definition for both the household survey and for the surveillance system might have captured more cases. For typhoid fever in particular, a broader case definition might have captured more cases among children <5 years of age who may experience milder illness (4) than adults. In addition, including infants <6 months of age would be important for measuring the incidence of infectious diseases that, unlike typhoid fever, occur frequently in this age group.

That healthcare providers enroll all eligible patients and use sterile venipuncture technique are vital to the success of this tool. We maximized enrollment by using a financial incentive and controlled blood culture contamination by active monitoring, regular feedback, and retraining health providers. Although we reduced blood culture contamination to 7% during the second half of the study, the overgrowth of contaminants may have prevented us from recovering pathogens from a proportion of blood cultures.

Implementing our surveillance tool in countries with a larger informal healthcare sector (e.g., traditional healers, informal pharmacists) would present challenges. These challenges would include identifying, training, and maintaining the participation of all healthcare providers. Implementing this surveillance system would also be difficult where a larger proportion of the population lacks access to any healthcare. Community use of antimicrobial agents was high among patients enrolled in our study, reflecting the global epidemic of community antibiotic abuse (20). Use of an antimicrobial agent before venipuncture is known to reduce the sensitivity of blood culture in the diagnosis of typhoid fever (6) and other infectious diseases, although we were not able to demonstrate this reduction with our data. For typhoid fever, we used the lower rather than higher reported sensitivity in blood culture to adjust our crude disease rates to account for community antibiotic use (6,21). In the future, data might be available to develop multipliers for the impact of antimicrobial agent use on blood culture sensitivity for specific infections. Furthermore, the epidemiology of typhoid fever within a country is likely to be heterogeneous in both time and location. Febrile illness surveillance should be replicated in several representative districts before making inferences about national disease incidence.

Our febrile illness surveillance tool could be applied in other countries and regions and lends itself to periodic and rapid implementation in multiple sites. For example, such sentinel surveillance could be conducted every 5 years in a region to update disease incidence assessments and to guide syndrome-based patient management. Potential applications extend beyond typhoid fever surveillance. The surveillance tool may provide a solution to the difficulties of measuring disease incidence that are faced for many causes of febrile illness (22). For example, because of asymptomatic parasitemia, a febrile event can be reliably attributed to malaria only when other causes of fever are excluded, a luxury not afforded to primary healthcare providers in developing countries and seldom available even in clinical malaria studies. Our model serves as a platform, whereby conducting additional tests (e.g., thick and thin blood smears for malaria parasites, acute- and convalescent-phase serologic tests) might permit the simultaneous measurement of the incidence of a broad range of causes of febrile illness. In so doing, the fraction of febrile illness attributable to various etiologic agents can be estimated simultaneously for a country or region. Collecting additional diagnostic specimens (e.g., urine) would permit the assessment of relevant biomarkers (e.g., the level of community antibiotic use) (23,24). The model likely could be integrated with sentinel surveillance for diseases such as typhoid, brucellosis, leptospirosis, malaria, and melioidosis, and for a range of viral and rickettsial diseases. The resulting data could be used to guide appropriate local modifications of algorithms for the empiric management of febrile persons (e.g., the fever module of the World Health Organization/United Nations Children's Fund guidelines for the integrated management of childhood diseases), especially in areas with low prevalence of malaria (25,26).

In Egypt, the febrile illness surveillance tool will be assessed in other districts to develop a national picture of the current incidence and causes of febrile illness. These data will help Egypt set priorities for spending for control measures and target specific prevention activities for a group of diseases that have thus far eluded accurate enumeration and standardized comparisons of incidence.

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Human Milk Secretory Antibodies against Attaching and Effacing *Escherichia coli* Antigens

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Secretory immunoglobulin A (sIgA) is a primary factor responsible for preventing attachment of enteropathogens to gut epithelium in breastfeeding infants. We compared the frequency of sIgA to major surface antigens of enterohemorrhagic *Escherichia coli* (EHEC) in milk of 123 women from the United States and Mexico to determine whether regional differences existed in the frequency of antibodies to these surface antigens. In both groups of women, milk commonly has sIgA against various EHEC lipopolysaccharides, EspA, EspB, intimin, and less frequently against Shiga toxin. The study suggests that persons living in the U.S. are exposed to attaching/effacing enteropathogens more frequently than is generally assumed. The low frequency of antibodies to Stx1 (in 12% of Mexican and in 22% of U.S. samples) suggests that the rare appearance of hemolytic uremic syndrome in adults is not due to neutralization of toxin at the gut level. Only anti-EspA is found in most milk samples from both populations of women. EspA may represent a useful target for an immunization strategy to prevent EHEC disease in humans.

Enterohemorrhagic *Escherichia coli* (EHEC) produces multiple virulence factors; the most important are protein synthesis–inhibiting toxins: Shiga toxin 1 (Stx1) and 2 (Stx2). EHEC causes nonbloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS). A large number of EHEC serotypes infect humans. In the United States, the predominant EHEC serotype associated with serious disease is *E. coli* O157:H7. HUS complicates approximately 5% to 8% of infections caused by *E. coli* O157:H7.

Virulence in EHEC reflects not only toxin production but also the pathogen's ability to colonize the gut. Colonization by EHEC is related to the pathogen's ability to form attaching and effacing lesions (intestinal mucosal changes seen in transmission electron microscopy and originally seen in intestines of animals infected with enteropathogenic *E. coli* [EPEC]) (1). The lesions are characterized by localized destruction of brush border microvilli and intimate adhesion of the bacterium to the host cell membrane. At the site of bacterial attachment, the host cell membrane forms a pedestal-like structure. Immunofluorescence microscopy has shown that the area of host cell in proximity to the bacterium contains polymerized actin, α-actinin, talin, and ezrin (2). In both EHEC and EPEC, the proteins that mediate this attachment are encoded in a chromosomal pathogenicity island called the "locus of enterocyte effacement" (LEE) (3). Secretion of LEE proteins is triggered by close contact with host cells. Once triggered to express LEE, the bacterium forms an export apparatus that includes a tube made of multimers of a protein (EspA); this surface organelle acts as a conduit between the bacteria and host cell (4–6). EspB, a protein thought to be involved in pore formation, is transferred to the host cell by this conduit and is found in both the host cell membrane and cytosol (7). EspB, with the aid of a second membrane lytic protein, EspD, forms pores in the host cell as part of the translocation mechanism (8). The EspA organelle is used to transfer the translocated intimin receptor (Tir), which is then inserted into the host cell membrane, where it binds to intimin, a bacterial outer membrane protein (9,10) and triggers the host cytoskeletal events that lead to attaching/effacing lesion formation.

Development of specific immunity to these antigens plays a role in protecting against infection. Immune responses are elicited in patients who are infected with EHEC or EPEC. Children infected with EPEC have been shown to have serum immunoglobulin (Ig) G against intimin, EspA, and EspB (11). Likewise, serum IgG against Tir, intimin, EspA, and EspB has been demonstrated during EHEC infection (12–14). Specific IgG against O157 lipopolysacchartide (LPS) (15) and against EHEC intimin (16) blocks adherence in vitro.

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Human milk is protective against many enteropathogens. Because antibodies in milk reflect previous immunologic events in the mother's gut, human milk is ideally suited for defining mucosal protective immunity. Lymphocytes are known to travel from the gut to the mammary gland. Human milk contains antibodies to EHEC intimin, EspA, EspB, and Tir (17–20), and to EPEC intimin (19). Incubation of colostrum or pooled human milk decreases EPEC adherence (21–23). Hence, the protective effect seen in vitro has been attributed to the presence of these antibodies. The role of virulence antigen–specific sIgA in protecting against EHEC has not been assessed directly. For EHEC and EPEC, as for most enteric pathogens, the best hope for disease control is through a vaccine strategy. Candidates for an EHEC vaccine might, in theory, include the surface-exposed components, secreted components, or both.

Antibodies in human milk can be used as an epidemiologic tool because antibodies reflect previous infection in the mother (24). We compared milk samples of women from Mexico and from the United States for antibodies to LPS and virulence proteins involved in the initial bacteria-host cell interaction. We also evaluated milk samples for antibodies to Stx1, a major secreted virulence factor of EHEC. We compared the frequency and amount of antibodies to each of the antigens in these two populations to determine whether important differences in sIgA antibodies exist that might provide insight into exposure to these antigens and potential protective mechanisms.

Methods

Population and Milk Collection

Human milk samples were collected after informed consent from 123 women living in two widely separated areas of North America: Mexico City and Norfolk, Virginia. None of the women had premature infants. The study was approved by the Institutional Review Boards of each participating institution. None of the women were known to have an underlying illness. Whether they had previously experienced infections with attaching/effacing organisms was unknown. Milk samples were obtained by using an Ameda Egnell pump (Hollister, Inc., Libertyville, IL). Samples were stored at –70°C after collection. Human milk samples were thawed and centrifuged at 13,200 rpm three times to obtain a clear fraction.

Preparation of Antigens

E. coli LPS

E. coli O157:H7 LPS was extracted with phenol water by using the method described by Westphal and Jann (25). Other purified LPS (O26, O55, O111, O127, and O128) were obtained from Sigma (Aldrich Corp., St. Louis, MO).

Stx

Stx1 was purified from *Shigella dysenteriae* serotype 1 as previously described (26). We evaluated antibodies to Stx1 rather than to Stx2 because approximately 90% of EHEC produce Stx1. Stx2 appears to be less immunogenic than is Stx1.

EspA and EspB

E. coli M15 with the plasmids encoding either C terminal histidine-tagged EspA or histidine-tagged EspB cloned from EHEC *E. coli* O26:H- strain 413/89-1 (6) was grown in Terrific broth (ENE Mate, ISC Bioexpress; Kaysville, UT) until optical density (OD) at A_{600} was 0.7. Bacteria were induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM at 30°C for 3 h. For purifying EspB, phenylmethylsulfonyl fluoride (1 mM) was added to the culture media. The cells were harvested by centrifugation. The resulting pellet was lysed through the addition of lysozyme and sonication. The resulting supernatant was mixed with nickel nitriloacetic agarose (Qiagen, Inc., Valencia, CA) for 1 h at 4°C. The agarose was then poured into a column and washed with increasing concentrations of imidazole in phosphate buffer (10 mM) to elute the purified proteins. All steps during the purification were performed under nondenaturing conditions following manufacturer's instructions. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to confirm purity of eluted proteins.

Intimin Gamma

The 281 amino acid C terminal (extracellular) portion of intimin ($C_{281}\gamma$) was cloned from *E. coli* O157:H7 strain 86-24 by using as forward primer 5-GATC- AAACCAAG-GCCAGCATTACTGAGATT and reverse primer 5-AATAATTATGCCC- CGACTAAAACA. The *taq* polymerase amplified segment was inserted into polymerase chain reaction T7 NT-TOPO so that six histidine residues were added to the N terminus. The sequence was verified by digestion with *Eco*RI and *Bam*HI and automated sequencing by using dye-terminator chemistry (BigDye as the fluorescent marker) in an ABI PRISM model 377 Genetic Analyzer (Applied Biosystems, Foster City, CA). The plasmid was then inserted into BL21(DE3)pLysS and expression inducted with IPTG. After partial purification with nickel nitriloacetic agarose chromatography, the amplified protein was detected on immunoblots (as described below). The $C_{281}\gamma$ was located on SDS-PAGE by size, intensity of the band after IPTC induction, and confirmation that the band contained 6 x His by Western blot.

Specific sIgA Determination by Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was used to determine the presence and amount of sIgA against Stx1, EspA, EspB, and each LPS. The amount of sIgA was estimated based on the mean of duplicate measures of OD of the antigen-coated wells minus the control background wells. For each ELISA all samples were run on the same day to eliminate day-to-day variation. For EspA, EspB, and each of the LPS, 96-well polystyrene plates were coated overnight at 4°C with 1 μg/well of each antigen in carbonate buffer (pH 9.6). After coating, plates were blocked with 200 μL of 5% bovine serum albumin (BSA) in 10 mM sodium phosphate-buffered saline (PBS) for 1 h at 37°C. After each step, plates were washed five times with PBS containing 0.05% Tween 80. Human milk samples diluted 1:20 in 1% nonfat dry milk in PBS were incubated at 37°C for 1 h. Goat anti-human sIgA conjugated to horseradish peroxidase (Cappel Division of Organon Teknika, Durham, NC) was added after washing. Hydrogen peroxide with o-phenylenediamine dihydrochloride was used for color development. The reaction was stopped by adding 2 N sulfuric acid, and plates were read at 490 nm. For Stx1, we used a variation of a previously described receptor binding ELISA (27). Polyvinyl chloride plates were coated with Gb3, blocked with 5% BSA-PBS, washed five times with PBS-Tween, and coated with 1 μg/well of toxin overnight at 4°C. The plates were washed five times with PBS-Tween and blocked with 5% BSA-PBS before 100 μL of milk sample per well diluted 1:20 with 1% nonfat dry milk in PBS was applied. Goat anti-human sIgA conjugated to horseradish peroxidase was added after washing and OD determined as above.

SDS-PAGE and Western Blot Analysis

SDS-PAGE was performed on the protein samples diluted in sample buffer (2-mercapthoethanol, SDS, and 0.1% bromophenol blue), boiled for 5 min, and loaded into 12.5% gels. The protein bands were visualized by staining with Coomassie blue. After SDS-PAGE, unstained protein antigens or LPS were transferred electrophoretically onto nitrocellulose membranes and blocked with 3% nonfat dry milk in Tris buffer for 1 h. The membranes were incubated overnight with the human milk samples diluted 1:20 with 1% nonfat dry milk in Tris buffer at 4°C. After three washes, the bound sIgA was detected by using peroxidase conjugates as above with chloro-1-naphthol as the color reagent. Western blot was used to determine the ELISA sensitivity and specificity as well as to detect sIgA to $C_{281}\gamma$.

Statistical Analysis

Chi-square or Fisher exact test was used to compare the frequency of sIgA-positive milk samples in the two populations for each antigen. Since anti-$C_{281}\gamma$ antibody was detected by immunoblot rather than ELISA, its relationship to other antibodies was determined by chi-square test or Fishers exact test. For other antibodies that were measured quantitatively, differences between the populations in amounts of antibodies were determined by a two-tailed Mann-Whitney test. The correlation between the amount of sIgA for different antigens was determined by linear regression of ELISA ODs. Because of the multiple comparisons made, differences were considered significant at $p \leq 0.01$.

Results

Description of Populations

Milk samples from 73 women in Mexico City and 50 women in Norfolk, Virginia, were studied. No difference existed in the timing of collection of the milk samples between the two populations (Table 1).

Validation of Assays

Purification of histidine-tagged EspA and EspB resulted in a single band of protein in the eluate as visualized by SDS-PAGE. As expected, ELISAs were highly reproducible. For example, the correlation between OD_{490} on repeat assays of randomly chosen samples for antibody to O111 was 0.858 ($p<0.001$), and for antibody to O26, the correlation was 0.807 ($p<0.001$). Based on the Western blot studies, the cutoff for a positive ELISA for each antigen was considered to be an $OD \geq 0.1$ at 490 nm. The sensitivity and specificity of the ELISA for various antigens were high as determined by using Western blot as the standard. For example, sensitivity and specificity were 82% and 71% for O157 LPS, 100% and 88% for EspB, and 93% and 100% for EspA, respectively. Because of the inherent differences in sensitivity of immunoblots compared to enzyme immunoassays on plastic plates, by using Western blot as the standard for defining ELISA cutoff, we may have underestimated somewhat the frequency of milk samples that contain sIgA to some antigens.

Prevalence of Antibodies to EHEC Antigens

Large variations occurred in frequencies of milk samples containing antibody to the various LPS. The percentages of milk samples that contained antibodies to various LPS types were similar in the two populations, with the exception of anti-O128, which occurred significantly more

Table 1. Distribution of milk samples over duration of lactation in the two study cohorts

No. days of lactation	Mexico (%)	United States (%)
≤5	16 (22)	11 (22)
6–30	43 (59)	30 (60)
>30	14 (19)	9 (18)
Total	73 (100)	50 (100)

often in milk samples from women from the United States (Table 2). However, the amount of antibody to the various LPSs differed significantly in the two settings. Mexican women had higher levels of antibodies (Table 3) against O55 and O127; they also had higher antibody levels against EspA and EspB. Milk samples from U.S. women had higher levels of antibodies against O26, O111, O128, and Shiga toxin.

Relationship of sIgA Antibodies to LSP, Intimin Gamma, EspA, EspB, and Shiga Toxin

Women in both populations who had antibodies to EspB nearly always had antibodies to EspA in their milk; 98% of those whose milk samples were positive for anti-EspB antibodies were also positive for anti-EspA antibodies, whereas those who were positive for anti-EspA were positive for anti-EspB 43% of the time ($p<0.01$ by chi square). Although anti-EspB was found less often than anti-EspA, the amount of anti-EspB correlated with the amount of anti-EspA in both populations (Tables 4 and 5). The amount of anti-EspA antibodies also was correlated with anti-O55 in milk samples from Mexico and with anti-O55 and anti-O127 milk samples from the United States. The data regarding antibodies to antigens that are EHEC-specific suggested that O55 and O111, but not O157, are important EHEC serotypes in Mexico (Table 4) since anti-Stx1 correlated well with antibodies to these LPS types. These findings are consistent with studies of meat samples in Mexico, which suggest that O157 is rarely found (28). Anti-$C_{281}\gamma$ correlated with anti-Stx1 and anti-O55 in Mexican women but not in U.S. women (Table 6). Most milk samples (17 [85%] of 20) positive for anti-$C_{281}\gamma$ were from Mexican women with antibody to O55 LPS ($p=0.0001$). These relationships imply that Shiga toxin and intimin gamma antibodies were linked to *E. coli* O55 infection. In the milk samples from U.S. women, anti-Stx did not correlate with any LPS type including O157 (Table 5).

Discussion

The specific antibodies that may be important in sIgA-mediated passive immune protection and infection-induced active immunity in human milk are not known. Milk, because it contains the infection-triggered active mucosal immune response of the mother, reflects antibodies that are relevant to clearing her particular infection and to subsequently protecting her infant. As such, milk antibodies indicate to which antigens the immune system has been most responsive. Focusing on such antigens may suggest candidates for vaccine development.

In EPEC, formation of the attaching/effacing lesion is central to pathogenesis. Colostrum, and in particular, the sIgA fraction, has been shown previously to inhibit localized adherence of EPEC (21,22). Epidemiologic data also support the importance of attaching/effacing lesion formation in the pathogenesis of EHEC. The *eae*A gene is more commonly found in human isolates than bovine EHEC isolates (29,30) and in isolates known to have caused severe human diseases (31), suggesting that proteins found in LEE are important virulence factors.

Table 2. Comparison of prevalence of secretory immunoglobulin A to enterohemorrhagic *Escherichia coli* antigens in milk samples collected from women from Mexico and the United States

Antigen	Mexico (%) (N=73)	United States (%) (N=50)	p value
O26	58 (79)	43 (86)	NS[a]
O55	35 (48)	16 (32)	NS
O111	44 (60)	31 (62)	NS
O127	42 (58)	19 (38)	NS
O128	7 (10)	20 (40)	<0.0002
O157	25 (34)	18 (36)	NS
Stx	9 (12)	11 (22)	NS
EspA	68 (93)	45 (90)	NS
EspB	32 (44)	14 (28)	NS
Intimin ($C_{281\gamma}$)	20 (27)	16 (32)	NS

[a]NS, not significant by chi-square test.

Previous studies of anti-attaching/effacing antibodies in human milk (17,19,22,32) have reported data on small numbers of milk samples, sometimes by using only pooled colostrum or by using crude antigens. The methodologic differences between previous studies and the current data are important to interpretating the data. The most comparable previous study in the literature is that of Parissi-Crivelli et al. (19). They found antibodies in the colostrum of 21 Mexican women against EspB in 57%, EspA in 76%, and intimin in 81%, compared with our findings of 44%, 93%, and 27%, respectively. The differences are important because the earlier data suggest that intimin is recognized much more frequently than our results show. Routine recognition of intimin would suggest that mucosal immunity that occurs naturally during infection targets this antigen. As such, intimin might be a useful antigen for a potential vaccine development plan. However, the difference between the studies may have occurred because we determined antibodies only to the extracellular C terminal 281-amino acid portion of intimin gamma that defines tissue tropism (33) rather than to the whole molecule. Antibodies to the intracellular portion of intimin were not detected by using our approach. That antibodies to the intracellular portion of intimin are relevant to protection is biologically implausible. In fact, antibodies to the N terminal two-thirds of the intimin molecule do not prevent EHEC from attaching to HEp2 cells (16). We have therefore focused on antibodies to the receptor-binding domain (34) that could be relevant to protection in the gut. This approach makes our observations more pertinent to intimin gamma-positive EHEC than to organisms that express other intimin variants.

Table 3. Comparison of quantity of anti-enterohemorrhagic *Escherichia coli* secretory immunoglobulin A in milk samples collected from women from Mexico and the United States [median and (range) optical density$_{490}$][a]

Antigen	Mexico (N=73)	United States (N=50)	p value
O26	0.143 (0.016–0.305)	0.203 (0.030–0.349)	<0.002
O55	0.096 (0–0.411)	0.057 (0–0.340)	<0.006
O111	0.126 (0–0.390)	0.143 (0–0.413)	NS
O127	0.124 (0–0.416)	0.072 (0–0.350)	<0.004
O128	0.026 (0–0.228)	0.079 (0.003–0.293)	<0.0000
O157	0.061 (0–0.470)	0.050 (0–0.260)	NS
Stx	0.027 (0–0.470)	0.043 (0–0.279)	<0.007
EspA	0.283 (0.063–0.666)	0.201 (0.015–0.490)	<0.005
EspB	0.071 (0–0.546)	0.021 (0–0.430)	<0.002

[a]Mann-Whitney test for differences in amounts of antibodies for the two populations; all samples tested at 1:20 dilution; NS, not significant.

This study supports the previous suggestion that human milk can be used as an epidemiologic tool (24). Because lymphocytes travel from the gut to mammary glands by the common mucosal immune system, sIgA in human milk reflects previous intestinal infection. Many insights into antigen-specific sIgA, the most relevant antibody to protection from intestinal infection, can be gained by studying human milk. For example, our data show regional variations in exposure to *E. coli* LPS types in two study sites. Exposure to multiple LPS types, including O55 and O111, correlates with anti-EspA in the United States, while in Mexico only O55 occurs commonly enough for anti-EspA to correlate with anti-LPS. The lack of correlation between the presence of antibodies against Stx1 and O157 LPS in the United States suggests that mucosal immunity to the toxin is not related to previous exposure to O157 EHEC. In Mexico, the primary stimulus for development of antibody to Stx1 may be becoming infected with O55 or O111 EHEC rather than with O157 EHEC. That these serogroups are infrequently associated with HUS suggests that they may be less virulent, less easily diagnosed, or less likely to cause outbreaks of disease than *E. coli* O157:H7. The lack of readily available screening methods for EHEC serotypes other than O157 may cause the frequency of non-O157 types to be underestimated. The surprisingly low frequency of sIgA against Shiga toxin suggests that mucosal immunity to the toxin is not the basis for the low frequency of HUS in adults; antibodies with expressed virulence factors that block attachment are probably more important.

We thought that milk samples from the U.S. women would rarely show evidence of immunity to antigens expressed by EPEC or EHEC. In fact, the data suggest that exposure to organisms that produce attaching/effacing lesions must be much more common than anticipated. Antibodies to surface antigens of EHEC, particularly those involved in the initial interaction of bacteria with intestinal epithelial cells, frequently are found in human milk. The data suggest that most women have been exposed to bacteria-expressing proteins that mediate the attaching/effacing phenotype, whether these women live in Mexico City or Norfolk, Virginia. Stool survey data also suggest that these infections may be occurring more often than commonly assumed. Bokete et al. analyzed stools from 445 children in the United States and found that 5.6% shed non-O157:H7 *eae*A+ *E. coli* (35). A multicenter prevalence study on the cause of outpatient pediatric nondysenteric diarrhea in the United States showed that 2.7% had *E. coli* with localized adherence phenotype or with a positive probe for EPEC (36). Most U.S. laboratories do not routinely evaluate pediatric diarrheal stools for the presence of EPEC or EHEC. The sIgA antibody data shown here, coupled with the stool survey data, suggest that organisms producing the attaching/effacing lesion must be common pathogens in the United States. The similarity between frequencies of antibodies to important surface antigens suggests that the prevalence of HUS in industrialized countries as opposed to developing countries (37) is not due solely to differences in frequency of exposure to EPEC.

Our studied showed that EspA was found in most milk samples (>90%), while Parissi-Crivelli found a much lower frequency (19). The difference may exist because of their definition of a positive ELISA for EspA; they arbitrarily set an OD >0.2 as positive, while we established a cutoff by immunoblot. The difference could also reflect the antigens used for ELISA. We used purified EspA, which was confirmed by SDS-PAGE to be a single band with no

Table 4. Correlations in amount of antibodies in human milk from women in Mexico to various enterohemorrhagic *Escherichia coli* antigens (correlation/p value)

Antigen	EspA	EspB	Stx1
EspB	0.405/<0.001		
Stx1	0.242/NS[a]	0.000/NS	
O26	0.161/NS	0.124/NS	0.195/NS
O55	0.303/<0.01	0.046/NS	0.310/<0.01
O111	0.235/NS	0.069/NS	0.358/<0.001
O127	0.164/NS	0.115/NS	0.232/NS
O128	0.291/NS	0.202/NS	0.002/NS
O157	0.056/NS	0.133/NS	0.131/NS

[a]NS, not significant.

Table 5. Correlations in amount of antibodies in human milk from women in the United States to various enterohemorrhagic *Escherichia coli* antigens (correlation/p value)

Antigen	EspA	EspB	Stx1
EspB	0.464/<0.001		
Stx1	0.277/NS[a]	0.182/NS	
O26	0.276/NS	0.186/NS	0.165/NS
O55	0.425/<0.01	0.304/NS	0.054/NS
O111	0.380/NS	0.330/NS	0.200/NS
O127	0.470/<0.001	0.056/NS	0.262/NS
O128	0.300/NS	0.002/NS	0.135/NS
O157	0.214/NS	0.142/NS	0.068/NS

[a]NS, not significant.

detectable contaminants, while Parissi-Crivelli used sonicates of organisms expressing an unknown amount of EspA on a plasmid and subtracted as background the sonicates of the vector bacteria lacking the gene for EspA. We studied titers at a 1:20 dilution. Although the relatively low titers detected could reflect exposure to related antigens produced by other bacteria, the immunoblots demonstrated that the antibodies did react with the specific antigens.

Why the immune system recognizes one antigen more often than another when both are expressed during infection is not clear. However, given current understanding of the virulence mechanism involved in producing attaching/effacing lesions, the secretory IgA data are readily understandable. The lower frequency of antibodies to EspB than to anti-EspA reflects that the immune system has better recognition of the multimeric surface-exposed EspA organelle. EspB, a protein that is directly injected into the cytoplasmic membrane of intestinal epithelial cells through the EspA organelle, is likely to be less available for uptake by antigen-presenting cells.

In summary, antibodies against LEE-encoded proteins were common in samples of human milk from our two groups. Because of the structural similarity among EspA variants from multiple pathogens and the high frequency of anti-EspA antibodies, cross-reactive antibodies against EspA may provide broad cross-protection against multiple serotypes. EspA is more conserved than other virulence antigens; most clone 1 EPEC have identical EspA types, while most clone 2 are nearly 95% identical (38). EspA in *E. coli* O127:H6 and *E. coli* O157:H7 are 85% identical (39) EspA is surface expressed and multimeric. Antibody to EspA is present more often than is antibody to other surface proteins. Natural exposure to EspA appears to elicit a good immune response that is long lasting as reflected by the high percentage of women who have anti-EspA in their milk samples. Unlike antibodies to LPS or intimin (40) that may protect against a very limited group of enteropathogens, antibodies to EspA might be able to block attachment by both EPEC and EHEC of many serogroups and thereby provide broad cross-protection. EspA may be a useful candidate for an immunization strategy that could lead to a vaccine that protects against both EHEC and EPEC of multiple serotypes.

Table 6. Relationship between presence of antibody to intimin gamma ($C_{281}\gamma$) and antibodies in human milk to various enterohemorrhagic *Escherichia coli* antigens[a]

Antigen	Mexican women	U.S. women
EspA	NS	NS
EspB	NS	NS
Stx1	<0.01	NS
O26	NS	NS
O55	<0.0001	NS
O111	NS	NS
O127	NS	NS
O128	NS	NS
O157	NS	NS

[a]p values shown for association with antibodies by chi-square analysis; NS, not significant.

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Aeromonas spp. and Traveler's Diarrhea: Clinical Features and Antimicrobial Resistance

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Traveler's diarrhea is the most common health problem of international travelers. We determined the prevalence of *Aeromonas* spp. associated with traveler's diarrhea and analyzed the geographic distribution, clinical features, and antimicrobial susceptibility. *Aeromonas* spp. were isolated as a cause of traveler's diarrhea in 18 (2%) of 863 patients. *A. veronii* biotype sobria was isolated in nine patients, *A. caviae* in seven patients, and *A. jandaei* and *A. hydrophila* in one patient each. *Aeromonas* spp. were isolated with a similar prevalence in Africa, Latin America, and Asia. Watery and persistent diarrhea, fever, and abdominal cramps were common complaints. All strains were resistant to ampicillin; showed variable resistance to chloramphenicol, tetracycline, and cotrimoxazole; and were susceptible to cefotaxime, ciprofloxacin, and nalidixic acid. The persistence of symptoms made antimicrobial treatment necessary.

Traveler's diarrhea is the main infectious disease reported in persons traveling abroad. Among the microorganisms responsible, bacteria represent approximately 61% (1). Enterotoxigenic *Escherichia coli*, enteroaggregative *E. coli,* and *Shigella* spp. are the most common bacteria involved (1,2). Other bacteria that cause diarrhea, such as *Salmonella, Campylobacter, Yersinia, Aeromonas,* and *Plesiomonas* spp., are isolated less often (1).

The genus *Aeromonas* has high diversity: at least 16 DNA hybridization groups are recognized (3). Among these genospecies, *A. hydrophila, A. caviae,* and *A. veronii* biotype sobria are considered of clinical significance (4,5). The spectrum of infectious diseases caused by *Aeromonas* species includes gastrointestinal infections as well as extraintestinal infections such as cellulitis, wound infections, septicemia, urinary tract infections, and hepatobiliary and ear infections, among others (6). Although healthy carriers of *Aeromonas* spp. have been described, several case-control studies have shown that these bacteria cause diarrhea (6). With the incorporation of genotypic techniques, identification of *Aeromonas* to species level has improved (7). The main objective of this study was to determine the prevalence of *Aeromonas* spp. as a cause of traveler's diarrhea and to analyze the species' geographic distribution, clinical features, and susceptibility to antimicrobial agents.

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Methods

Patients

A total of 863 patients with traveler's diarrhea were recruited from the Tropical Medicine Unit of the Hospital Clinic of Barcelona, Spain, during the period January 1999–December 2001. All patients completed an epidemiologic questionnaire; their clinical history was taken, and a physical examination was performed. Traveler's diarrhea was defined as the occurrence of three or more episodes of watery stool within a 24-hour period, with or without other symptoms, or the occurrence of unformed stools accompanied by one of the following: vomiting, nausea, abdominal cramps, fever, chills, prostration, or tenesmus. Persistent diarrhea was defined as that of >14 days' duration.

Microbiologic Tests

A stool sample was collected, sent to the Laboratory of Clinical Microbiology, and processed for bacterial, viral, and parasitologic studies. To isolate *Aeromonas* spp., blood agar supplemented with ampicillin and a selective media, CIN (cefsulodin-irgasan-novobiocin) agar, were used. After incubation at 37°C for 24–48 hours, an oxidase test was performed on the colonies compatible with bacilli. Biochemical criteria were used to identify *Aeromonas.* Identification of the species was performed by 16S rDNA–restriction fragment length polymorphism as previously described (7,8). The biotype of *A. veronii* strains was identified on the basis of a positive reaction to arginine dihydrolase and negative response to bilis-esculin hydrolysis and production of ornithine decarboxylase (9).

Susceptibility Testing

Antimicrobial susceptibility tests were performed by using an agar disk diffusion method advocated by the

National Committee for Clinical Laboratory Standards (10). Antimicrobial disks (ampicillin 10 µg; cefotaxime 30 µg; chloramphenicol 30 µg; ciprofloxacin 5 µg; nalidixic acid 30 µg; tetracycline 30 µg; and trimethoprim-sulfamethoxazole 1.25/23.75 µg) were obtained from Becton Dickinson (Cockeysville, MD). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality-control strains.

Results

Distribution and Geographic Origin of Species Causing Traveler's Diarrhea

Aeromonas spp. were isolated as a cause of traveler's diarrhea in 18 (2%) of 863 patients. *A. veronii* biotype sobria was isolated in nine patients, *A. caviae* in seven patients, and *A. jandaei* and *A. hydrophila* in one patient each (Table 1). In three of these patients, another enteropathogen was also found: in one patient who had traveled to Mexico, *Shigella sonnei* was isolated together with *A. veronii*; in another patient traveling to India, *Giardia lamblia* was detected together with *A. veronii*; in the third patient, who had traveled to Thailand, *Salmonella* Typhimurium was found with *A. veronii*. The frequency of *Aeromonas* spp. as a cause of traveler's diarrhea was similar in patients returning from Africa (1.7%), Latin America (1.8%), and Asia (2.3%) (Table 1).

Clinical Features

The signs and symptoms of *Aeromonas* enteritis in these 18 patients are summarized in Table 2. Sixteen of the 18 patients had watery diarrhea; these were the cases associated with *A. veronii* biotype sobria and *A. caviae.* The patients with enteritis caused by *A. hydrophila* and *A. jandaei* had loose stools. Fifty percent of the patients had fever and abdominal cramps, whereas nausea and vomiting were uncommon complaints. Gross blood was observed in the stools of one patient, but this could be attributed to the *S. sonnei* isolated in the same stool. In 9 of the 18 patients, diarrhea was persistent.

Antimicrobial Susceptibility

The antimicrobial susceptibility of *Aeromonas* spp. isolates causing traveler's diarrhea is shown in Table 3. All strains were resistant to ampicillin but susceptible to cefotaxime, ciprofloxacin, and nalidixic acid. The susceptibility to chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole varied. Some 66.6% of *A. veronii* biotype sobria strains and 71.4% of *A. caviae* strains were susceptible to chloramphenicol; 55.6% of *A. veronii* biotype sobria strains and 71.4% of the *A. caviae* strains were susceptible to tetracycline; and 77.8% of *A. veronii* biotype sobria and 100% of *A. caviae* were susceptible to trimethoprim-sulfamethoxazole.

Treatment

While travelling, two patients received treatment, amoxicillin in one case and amoxicillin plus clavulanic acid in the other. Patients with persistent diarrhea were treated with the following antibiotics: norfloxacin (one patient), ciprofloxacin (six patients), and trimethoprim-sulfamethoxazole (two patients); all recovered.

Discussion

In this study we describe the prevalence of different types of *Aeromonas* species associated with traveler's diarrhea in a cohort of travelers to a variety of tropical and subtropical countries. In contrast, other published studies have often been selective in terms of the types of travelers, geographic areas visited, or attempts to isolate specific microorganisms with the aim of testing antibiotic efficiency. In our study, *Aeromonas* spp. were isolated in 18 (2%) of 863 patients with traveler's diarrhea. *A. veronii* biotype sobria and *A. caviae* were the most frequently isolated species. These findings agree with the results of Hänninen et al. (11), who reported that these were the most common

Table 1. Species and geographic distribution of clinical isolates of *Aeromonas* spp. causing traveler's diarrhea

Geographic area	*A. veronii* biotype sobria (n=9)	*A. caviae* (n=7)	*A. jandaei* (n=1)	*A. hydrophila* (n=1)
Guatemala	1	1		
India	2	1[a]	1[a]	1
Iran		1		
Kenya	1			
Mali/Burkina Faso	1			
Mexico	2[b]			
Nicaragua		1		
Paraguay		1		
Sahara	1			
Senegal		2		
Thailand	1			

[a]This patient traveled to India and Nepal.
[b]One traveler also visited Guatemala.

Table 2. Clinical features of patients with traveler's diarrhea associated with *Aeromonas* spp.

Sign or symptom	No. of patients with symptoms/ total patients (n=18) *A. veronii* biotype sobria	*A. caviae*	Overall[a]
Watery diarrhea	9/9	7/7	16/18
Abdominal cramps	6/9	3/7	10/18
Persistent diarrhea	3/9	5/7	9/18
Fever	6/9	3/7	10/18
Nausea, vomiting, or both	2/9	0/7	3/18
Gross blood in stools	1[b]/9	0/7	1/18

[a]Also includes *A. hydrophila* and *A. jandai.*
[b]In this patient, a *Shigella sonnei* strain was also isolated.

Aeromonas spp. associated with traveler's diarrhea in tourists traveling to Morocco. Likewise, Yamada et al. (12) found that *A. veronii* biotype sobria was the *Aeromonas* species most frequently implicated as a cause of traveler's diarrhea in Japanese travelers returning from unindustrialized countries. In our study, the geographic distribution of *Aeromonas* species did not favor any predominant area: species were isolated with a similar prevalence in Africa, Latin America, and Asia. However, all four species (*A. veronii* biotype sobria, *A. caviae, A. jandaei,* and *A. hydrophila)* were isolated from patients returning from India. In India, *Aeromonas* spp. has been identified as an enteric pathogen in 1.8% of patients with diarrhea (13). In a recent study performed in Dhaka (Bangladesh), *Aeromonas* spp. were significantly associated with diarrhea, similar to occurrences in other countries (14–17).

In our study, 3 (16.7%) of the *Aeromonas* isolates were detected together with other enteropathogens. This situation allowed us to consider that the symptoms we observed in the patients with traveler's diarrhea associated with *Aeromonas* spp. were due to the presence of this *Aeromonas* organisms. In our study, watery stools, fever, and abdominal cramps were the most common symptoms, which is consistent with other reports (11,18). Albert et al. (18) suggested that isolates of *Aeromonas* spp. positive for both the *alt* and *ast* genes, which encode enterotoxins, were associated with watery diarrhea but that isolates positive only for the *alt* gene were associated with loose stools.

Table 3. Antimicrobial susceptibility of *Aeromonas* spp. causing traveler's diarrhea

Antimicrobial agent	No. of isolates showing susceptibility *A. veronii* biotype sobria (n=9)	*A. caviae* (n=7)
Ampicillin	0	0
Cefotaxime	9	7
Chloramphenicol	6	5
Ciprofloxacin	9	7
Nalidixic acid	9	7
Tetracycline	5	5
Trimethoprim/ sulfamethoxazole	7	7

Fifty percent of the patients with *Aeromonas* spp. enteritis had persistent diarrhea. Chronic diarrhea lasting more than 1 year caused by *A. caviae* has been reported (2). A direct link between drinking water and food contaminated with *Aeromonas* spp. and gastrointestinal disease has been demonstrated (19).

Patients with prolonged enteritis required treatment. A quinolone was the drug of choice, although increased occurrence of quinolone-resistant *Aeromonas* spp. strains has been reported in industrialized countries (20,21). Regarding the ß-lactam antibiotics, *Aeromonas* spp. strains analyzed in this study were, as expected, uniformly resistant to ampicillin, whereas third-generation cephalosporins, such as cefotaxime, showed good activity. These results are in accordance with those reported by other authors, showing that third-generation cephalosporins are active against *Aeromonas* spp. (2,22). The percentage of strains with resistance to chloramphenicol, tetracycline, or trimethoprim-sulfamethoxazole ranged from 22.9% to 45%. These levels of resistance are likely related to the extensive use of these antimicrobial agents in unindustrialized countries.

In summary, *A. veronii* biotype sobria and *A. caviae* are the *Aeromonas* species most frequently associated with traveler's diarrhea; watery diarrhea, fever, and abdominal cramps are the predominant clinical features. The persistence of symptoms makes the use of antimicrobial treatment necessary.

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Endemic, Notifiable Bioterrorism-Related Diseases, United States, 1992–1999

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Little information is available in the United States regarding the incidence and distribution of diseases caused by critical microbiologic agents with the potential for use in acts of terrorism. We describe disease-specific, demographic, geographic, and seasonal distribution of selected bioterrorism-related conditions (anthrax, botulism, brucellosis, cholera, plague, tularemia, and viral encephalitides) reported to the National Notifiable Diseases Surveillance System in 1992 to 1999. Tularemia and brucellosis were the most frequently reported diseases. Anthrax, plague, western equine encephalitis, and eastern equine encephalitis were rare. Higher incidence rates for cholera and plague were noted in the western United States and for tularemia in the central United States. Overall, the incidence of conditions caused by these critical agents in the United States is low. Individual case reports should be considered sentinel events. For potential bioterrorism-related conditions that are endemic and have low incidence, the use of nontraditional surveillance methods and complementary data sources may enhance our ability to rapidly detect changes in disease incidence.

In 2001, anthrax cases associated with the intentional distribution of *Bacillus anthracis* spores through the postal system re-emphasized that the deliberate exposure of humans to biologic agents can happen in the United States (1,2). Before the 2001 bioterrorism-associated anthrax events, terrorist attacks (e.g., the bombings of the World Trade Center in New York City in 1993, the Federal Building in Oklahoma City in 1995, and the Olympic Games in Atlanta in 1996; and an increase in intentional anthrax exposure hoaxes [3]) had already created substantial media and public attention because they highlighted our susceptibility to domestic terrorism, including bioterrorism. In addition, smaller focused acts of bacteriologic criminal assault had occurred in the United States, including the intentional contamination of salad bars with *Salmonella* organisms in 1984 in Oregon (4) and of muffins and pastries with *Shigella* organisms in Texas in 1996 (5); these acts served as a wake-up call announcing the threat of domestic bioterrorism. All of these events led the United States to revisit and update a national plan for bioterrorism preparedness and response in the late 1990s. In defining the role of the public health community in the detection of and response to bioterrorism, the Centers for Disease Control and Prevention (CDC) identified 10 major areas of need. One of these areas is ensuring reliable and timely disease surveillance and reporting to detect and investigate outbreaks (6).

In response to global bioterrorism threats, CDC has proposed a list of critical biologic agents that have potential for use in a terrorist incident (6–9). This list includes a wide range of biologic agents and prioritizes pathogens into three categories on the basis of their potential to affect the public's health, their potential for dissemination, and special needs for effective public health intervention. Prioritization of bioterrorism "threat" agents facilitates coordinated planning efforts for preparedness and response to bioterrorism at the local, state, and federal levels.

Using this guidance, public health systems can address the threat of bioterrorism by increasing healthcare sector awareness of and surveillance for these bioterrorism-related agents and the diseases they cause (10). In the United States, public health surveillance for conditions caused by the identified critical biologic agents is conducted in multiple ways. Although data regarding these agents are reported to different national surveillance systems at CDC, no single system is specifically designed for conducting surveillance for all bioterrorism-related agents or conditions. However, many states have routinely conducted surveillance for some of these conditions and report incidence data to CDC's National Notifiable Diseases Surveillance System (NNDSS) each week (Table 1).

We describe disease-specific trends in demographic characteristics and geographic and seasonal distribution of selected conditions caused by critical biologic agents reported to NNDSS. These diseases and conditions include anthrax, botulism, brucellosis, cholera, plague, tularemia, and selected viral encephalitides. By identifying patterns of endemic disease associated with critical agents, we

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Table 1. Number of reported cases and number of states reporting conditions caused by critical biological agents, National Notifiable Diseases Surveillance System, United States, 1992–1999[a,b]

Y	Anthrax	Botulism, foodborne	Botulism, Other[c]	Brucellosis	Cholera	Encephalitis, eastern equine	Encephalitis, western equine	Plague	Tularemia
1992–1999									
Total cases	1	223	148	813	223	29	1	77	885
1992									
Cases	1	21	4	105	103	N	N	13	159
States reporting	1	8	2	22	12	N	N	7	26
States requiring reporting	52	52	52	52	52	N	N	52	52
1993									
Cases	0	27	5	120	25	N	N	11	132
States reporting	0	10	3	23	11	N	N	4	24
States requiring reporting	52	52	52	52	52	N	N	52	52
1994									
Cases	0	50	8	119	39	N	N	17	96
States reporting	0	11	1	21	14	N	N	5	29
States requiring reporting	52	52	52	52	52	N	N	52	51
1995									
Cases	0	24	19	98	23	1	0	9	117
States reporting	0	9	4	24	14	1	0	4	24
States requiring reporting	52	52	52	52	52	U	U	51	N
1996									
Cases	0	25	22	112	4	5	0	5	88
States reporting	0	10	4	29	4	4	0	3	24
States requiring reporting	52	52	51	52	52	U	U	52	N
1997									
Cases	0	31	22	98	6	14	0	4	101
States reporting	0	8	5	26	5	6	0	3	24
States requiring reporting	50	52	51	51	51	U	U	51	N
1998									
Cases	0	22	29	79	17	4	0	9	96
States reporting	0	6	2	27	7	4	0	4	22
States requiring reporting	52	52	51	50	52	48	48	50	U
1999									
Cases	0	23	39	82	6	5	1	9	96
States reporting	0	8	5	18	5	2	1	2	27
States requiring reporting	52	52	52	51	52	48	49	51	U

[a]Abbreviations used: N, not nationally notifiable; U, unknown.
[b]Reports from 50 U.S. states, Washington, D.C., and New York City.
[c]Includes wound and unspecified botulism.

establish a baseline against which future disease incidence can be compared. This process should allow easier identification of unusual reports of disease incidence, which in turn will enhance the ability of the public health community to identify and investigate outbreaks.

Methods

Data and Sources

We analyzed NNDSS data voluntarily reported to CDC from state health departments from 1992 to 1999 (11). As of 1999, a total of 56 infectious diseases or conditions with public health surveillance case definitions (12,13) were considered nationally notifiable, as agreed upon by the Council of State and Territorial Epidemiologists and CDC (14). Each year, the Council and CDC review the list of nationally notifiable infectious diseases to determine whether conditions should be added or removed as new pathogens emerge or disease incidence changes (15). Based on state-specific health priorities, each state independently determines which of the nationally notifiable diseases should be made notifiable (i.e., legally reportable by healthcare providers or laboratories to the public health system within their jurisdiction). As a result, not all nationally notifiable diseases are legally reportable in all states. With some variation by jurisdiction, the completeness of public health surveillance is dependent on healthcare providers and laboratories submitting disease incidence or laboratory reports to local and county health departments, who then forward reports to the state health departments (16). Each week, health departments in 50 states, New York City (a separate reporting jurisdiction from New York State), the District of Columbia, and 5 U.S. territories compile surveillance data from their reporting sites and volun-

tarily transmit disease incidence data to CDC through the National Electronic Telecommunications System for Surveillance.

Conditions associated with critical biologic agents that were nationally notifiable, reported to NNDSS, and included in this study were anthrax, botulism, brucellosis, cholera, plague, tularemia, and selected viral encephalitides. Botulism is reported as two distinct conditions: foodborne botulism and other or unspecified forms of botulism, including wound botulism. All of the study conditions, except tularemia and selected viral encephalitides, were designated as nationally notifiable throughout the study period. Other than tularemia, only cases reported for those diseases designated as nationally notifiable and from states in which the disease was legally reportable were analyzed. Although tularemia was deleted from the nationally notifiable disease list in 1995 because of decreasing incidence, the disease remained reportable in most states, and the annual number of cases reported to NNDSS remained stable in subsequent years; therefore, tularemia incidence data for the entire study period were included in the analysis.

Analysis

Incidence rates were calculated for the demographic and geographic descriptors of sex, age (grouped as <1 year, 1–4, 5–14, 15–24, 25–39, 40–64, and ≥65 years), racial category (American Indian or Alaska Native, Asian or Pacific Islander, black, white, and other), Hispanic ethnicity, and state of residence. Seasonal incidence (spring, summer, fall, and winter) was examined on the basis of data reported with one of three types of dates: onset date, date of diagnosis, or date of laboratory result.

Average annual age-, sex-, race-, ethnicity-, and state-specific disease incidence rates for the period 1992–1999 were estimated by averaging the total annual number of case counts by subcategory, and dividing by the study's mid-year (1995) U.S. population. State-specific annual incidence rates were calculated by using postcensus estimates for July 1, 1992, through July 1, 1998, and population projections for 1999 from the U.S. Bureau of the Census. Incidence rates were calculated per 1 million population because of the small number of cases reported to NNDSS during the study period. Rates were not calculated for extremely rare conditions (anthrax and western equine encephalitis) or for conditions for which data were not collected in all years in the study period (eastern equine encephalitis). Data from U.S. territories were excluded in the analysis.

To provide an example of how historical disease incidence data may be used to assess the likelihood of a reported incident case in the future, we estimated the probability that a given reported case would have the distribution of age, sex, race, ethnicity, geographic residence, and season occurrence using the following formula: P(case) = P(age) x P(sex) x P(race) x P(ethnicity) x P(geographic residence) x P(season). The probability is derived from the NNDSS surveillance data and is calculated under the assumption that these demographic and geographic variables are independent.

Results

Disease reports for seven conditions caused by critical biologic agents were available for analysis by using NNDSS data for 1992 through 1999 (Table 1). The number of reported cases and incidence rates of the diseases examined in this study, excluding botulism and eastern equine encephalitis, declined or remained stable in the United States during the study period. Tularemia and brucellosis were the most frequently reported diseases (111 and 102 cases/year on average, respectively, yielding the highest estimated incidence rates of 42.1 and 38.7 cases/1 million persons/year, respectively). The least commonly reported diseases were anthrax, with only one case reported in 1992, and western equine encephalitis, with one case reported in 1999.

In general, sex-specific incidence rates were higher among male patients than among female patients for most study diseases. However, rates for foodborne botulism were higher among female than among male patients (Table 2). The age-specific incidence rates varied by disease. Most reported cases of study diseases were in persons ≥25 years of age; the exceptions were tularemia (highest rates were in children 1–14 years of age) and foodborne botulism (highest rates were in infants <1 year of age).

Race and ethnicity information was incompletely reported in NNDSS. More than 50% of reported cases of unspecified forms of botulism and cholera lacked information regarding race. Disease incidence varied among racial groups. High incidence rates for foodborne botulism, plague, and tularemia were identified in American Indians or Alaska Natives, and the highest incidence rates for cholera and infant botulism were identified in Asian or Pacific Islanders. The average annual disease-specific incidence rates for Hispanic persons were higher than the rates for non-Hispanic persons for most study diseases; the exceptions were plague and tularemia. Tularemia and plague had apparent seasonal patterns: >50% of cases occurred in the summer months (June, July, August). Almost half of reported cholera cases occurred in the winter season (December, January, February) (Figure, Table 2).

Table 3 lists the conditions caused by critical biologic agents in rank order by number of reported cases and incidence rates by state of residence; Table 4 gives the geographic region of residence for case-patients. Plague and

Table 2. Reported cases of conditions caused by critical biologic agents, by demographic characteristics and seasonal occurrence, National Notifiable Disease Surveillance System, United States, 1992–1999[a,b]

Demographic characteristics	Botulism, foodborne		Botulism, other[c]		Brucellosis		Cholera		Plague		Tularemia[d]	
	Cases (%)	Rate[e]	Cases (%)	Rate	Cases (%)	Rate	Cases (%)	Rate	Cases (%)	Rate	Cases (%)	Rate
Sex												
Male	101 (45.3)	9.8	86 (58.1)	8.4	487 (59.9)	47.4	82 (36.8)	8.0	41 (53.2)	4.0	587 (66.3)	57.1
Female	120 (53.8)	11.2	61 (41.2)	5.7	316 (38.9)	29.4	83 (37.2)	7.7	32 (41.6)	3.0	291 (32.9)	27.1
Sex not stated	2 (0.9)	NC	1 (0.7)	NC	10 (1.2)	NC	58 (26.0)	NC	4 (5.2)	NC	7 (0.8)	NC
Age group (y)												
<1	21 (9.4)	68.7	3 (2.0)	9.8	8 (1.0)	26.2	1 (0.4)	3.3	0 (0.0)	C	5 (0.6)	16.4
1–4	1 (0.4)	0.8	2 (1.4)	1.6	34 (4.2)	27.1	7 (3.1)	5.6	3 (3.9)	2.4	100 (11.3)	79.6
5–14	9 (4.0)	3.0	1 (0.7)	0.3	94 (11.6)	31.0	4 (1.8)	1.3	10 (13.0)	3.3	189 (21.4)	62.3
15–24	15 (6.7)	5.2	3 (2.0)	1.0	150 (18.5)	51.8	13 (5.8)	4.5	10 (13.0)	3.5	59 (6.7)	20.4
25–39	45 (20.2)	8.9	59 (39.9)	11.7	231 (28.4)	45.7	40 (17.9)	7.9	17 (22.1)	3.4	128 (14.5)	25.3
40–64	88 (39.5)	15.2	75 (50.7)	12.9	229 (28.2)	39.5	71 (31.8)	12.2	23 (29.9)	4.0	243 (27.5)	41.9
≥65	36 (16.1)	13.4	4 (2.7)	1.5	58 (7.1)	21.6	30 (13.5)	11.2	13 (16.9)	4.8	141 (15.9)	52.5
Age not stated	8 (3.6)	NC	1 (0.7)	NC	9 (1.1)	NC	57 (25.6)	NC	1 (1.3)	NC	20 (2.3)	NC
Race												
White	110 (49.3)	6.3	49 (33.1)	2.8	415 (51.0)	23.8	74 (33.2)	4.2	46 (59.7)	2.6	602 (68.0)	34.5
Black	2 (0.9)	0.8	5 (3.4)	1.9	53 (6.5)	20.0	3 (1.3)	1.1	0 (0)	NC	24 (2.7)	9.1
American Indian or Alaska Native	72 (32.3)	399.3	0 (0)	NC	1 (0.1)	5.6	0 (0)	NC	23 (29.9)	127.6	89 (10.1)	493.6
Asian or Pacific Islander	2 (0.9)	2.7	0 (0)	NC	10 (1.2)	13.3	21 (9.4)	28.0	0 (0)	NC	2 (0.2)	2.7
Other	1 (0.4)	NC	0 (0)	NC	7 (0.9)	NC	3 (1.3)	NC	0 (0)	NC	0 (0)	NC
Race not stated	36 (16.1)	NC	94 (63.5)	NC	327 (40.2)	NC	122 (54.7)	NC	8 (10.4)	NC	168 (19.0)	NC
Ethnicity												
Hispanic	29 (13.0)	13.3	53 (35.8)	24.3	468 (57.6)	214.5	81 (36.3)	37.1	7 (9.1)	3.2	12 (1.4)	5.5
Non-Hispanic	113 (50.7)	6.0	66 (44.6)	3.5	143 (17.6)	7.6	56 (25.1)	3.0	62 (80.5)	3.3	407 (46.0)	21.6
Ethnicity not stated	81 (36.3)	NC	29 (19.6)	NC	202 (24.8)	NC	86 (38.6)	NC	8 (10.4)	NC	466 (52.7)	NC
Seasonal occurrence[f,g]												
Spring	49 (22.0)	x	26 (17.6)	x	220 (27.1)	x	38 (17.0)	x	19 (24.7)	x	244 (27.6)	x
Summer	33 (14.8)	x	33 (22.2)	x	215 (26.4)	x	37 (16.6)	x	35 (45.5)	x	417 (47.1)	x
Fall	35 (15.7)	x	48 (32.4)	x	129 (15.9)	x	32 (14.3)	x	12 (15.6)	x	97 (11.0)	x
Winter	25 (11.2)	x	37 (25.0)	x	142 (17.5)	x	96 (43.0)	x	2 (2.6)	x	52 (5.9)	x
Eligible date not reported	81 (36.3)	x	4 (2.7)	x	107 (13.2)	x	20 (9.0)	x	9 (11.7)	x	75 (8.5)	x
Total	223 (100)	10.6	148 (100)	7.0	813(100)	38.7	223 (100)	10.6	77 (100)	3.7	885 (100)	42.1

[a]Abbreviations used: NC, not calculable; x, rate not calculated.
[b]Reports from 50 U.S. states, Washington D.C., and New York City.
[c]Includes wound and unspecified botulism.
[d]Not nationally notifiable 1995–1998.
[e]Average annual incidence rate.
[f]Includes data reported using one of the following date types only: onset date, date of diagnosis, or date of laboratory result.
[g]Spring includes March, April, and May; summer includes June, July, and August; fall includes September, October, and November; winter includes December, January, and February.

tularemia incidence demonstrated marked geographic distribution patterns. The highest incidence rates and number of cases of plague (86% of total plague cases) were reported from the mountain region (Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah, and Nevada); the highest incidence rates and number of cases of tularemia (78% of total tularemia cases) were reported from states in the mountain and the west central regions of the United States. In addition, >60% of botulism case-patients resided in the Pacific region. However, for most other conditions, the states reporting the highest number of cases did not have the highest incidence rates by place of residence. One exception was Alaska, which reported over twice the number of cases and almost 20 times the incidence rate for foodborne botulism compared with the states with the next highest case counts and incidence rates.

Tables 2–4 show descriptive NNDSS disease incidence data with which to estimate the probability that a reported incident case with selected demographic, geographic, and seasonal characteristics would occur. For example, if the

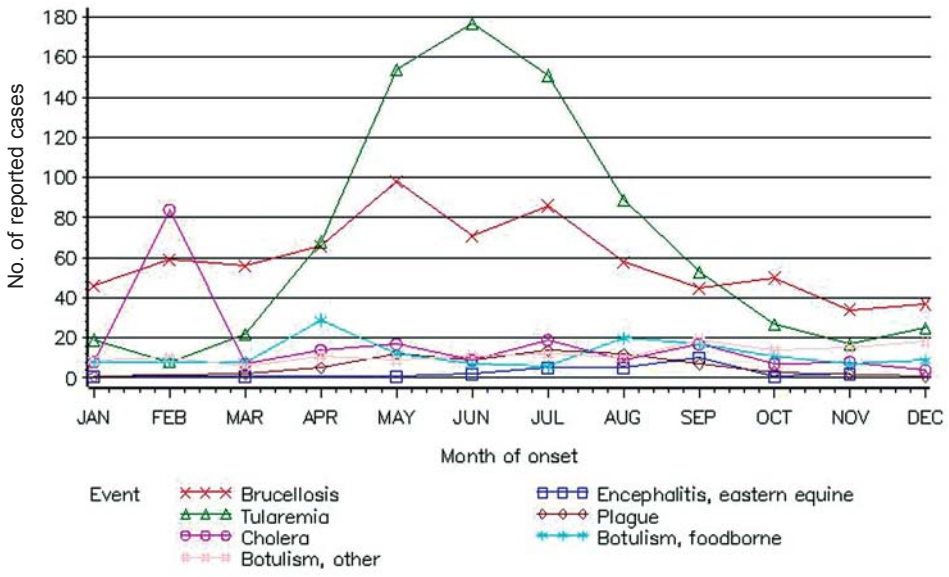

Figure. Reported cases of conditions caused by critical biologic agents, by month of onset, National Notifiable Disease Surveillance System, United States, 1992–1999. Cases are reported with one of the following types of dates: onset date, date of diagnosis, or date of laboratory result. Reports are from the 50 U.S. states, Washington, D.C., and New York City.

next reported case of brucellosis is in a 30-year-old non-Hispanic white man residing in Florida and occurs in the summer, under the assumption that these studied variables are independent, the probability of occurrence of this case would be 0.02% [P (brucellosis case-patient 1)=P (page 25–39) × P (non-Hispanic) × P (white) × P (male) × P (Florida) × P (summer)=P (28.4%) × P (17.6%) × P (51%) × P (59.9%) × P (3.8%) × P (30.5%) = 0.015%]. Similarly, if the next two reported tularemia case-patients are a 50-year-old non-Hispanic white man in the West South Central United States with onset in the summer (case-patient 1) and a 20-year-old non-Hispanic black woman in the West South Central region with onset in the summer (case-patient 2), then P (tularemia case-patient 1) = 0.86%, and an analogous calculation could be made for the subsequent case, P (tularemia case-patient 2) = 0.004%. Therefore, the probability that those two cases would have the observed characteristics would be P (cases 1 and 2) = P (case-patient 1) x P (case-patient 2) = 0.86% x 0.004%= 3.4^{-07}.

Discussion

Early detection of and response to a bioterrorist attack are crucial to decrease illness and deaths, especially in the event of a covert attack with a biologic agent (17). To accurately identify unusual or aberrant events prospectively among reports to NNDSS, we characterized the baseline, or endemic, disease incidence. These baseline data can be used by healthcare providers and public health department staff to compare endemic disease distributions and future reported disease incidence in their jurisdictions. From 1992 through 1999, all diseases caused by critical bioterrorist agents occurred at very low incidence rates in the United States. The most common diseases, tularemia and brucellosis, had only approximately 100 cases per year reported to NNDSS. Therefore, each case report of any of these conditions should be considered a sentinel event. Anthrax, eastern equine encephalitis, western equine encephalitis, and plague are so rare that even one case of these diseases should elicit immediate public health investigation and action.

Even with such low incidence, we identified patterns in disease incidence that better prepare us to identify potential bioterrorism events. In this analysis, certain diseases appear to be endemic in certain geographic areas (e.g., foodborne botulism in Alaska, brucellosis and plague in the western states, and tularemia in the central United States). Sporadic disease incidence outside of these regions might indicate aberrant activity. Similarly, certain diseases were common among certain demographic groups. For example, our study indicated a high cholera incidence rate in Asians or Pacific Islanders and a high botulism incidence rate in American Indians and Alaska Natives. Higher incidence rates for brucellosis and tularemia occurred in men and person $25 years of age (18). Reports of cases clustered in different demographic groups might suggest unusual disease activity potentially associated with bioterrorism or an opportunity for targeted prevention activities.

An explanation of these identified disease incidence patterns becomes clear when we examine disease-specific literature. Since 1989 and before the recent bioterrorism-related anthrax events, only one case of anthrax was reported in the United States, a marked decrease from a yearly average of 130 cases in the early 20th century (19–21). The decline in human disease caused by the critical agents is believed to have directly resulted from decreased incidence of animal diseases associated with

Table 3. Conditions caused by critical biologic agents, ranking by number of reported cases and incidence rates (per 1 million population) by state of residence, National Notifiable Disease Surveillance System, United States,[a] 1992–1999

	Rank by reported cases			Rank by incidence rate		
Disease	Rank	State	No. of cases	Rank	State	Average annual incidence rate
Botulism, foodborne						
	1	Alaska	72	1	Alaska	1,493.7
	2	Washington	33	2	Washington	75.7
	3	Texas	27	3	Idaho	75.0
	4	California	25	4	Wyoming	26.1
	5	Idaho	7	5	Colorado	20.0
Botulism, other[b]						
	1	California	128	1	D.C.	51.5
	2	New Mexico	3	2	California	42.1
	3	NYC	3	3	New Mexico	25.4
	4	D.C.	2	4	Mississippi	10.6
	5	Mississippi	2	5	Utah	7.3
Brucellosis						
	1	California	215	1	Wyoming	156.5
	2	Texas	200	2	Texas	133.0
	3	N. Carolina	58	3	N. Carolina	100.7
	4	Illinois	53	4	Iowa	92.3
	5	Florida	31	5	Arizona	87.1
Cholera						
	1	California	115	1	Nevada	130.4
	2	Nevada	16	2	California	45.5
	3	Texas	14	3	Hawaii	42.4
	4	Louisiana	7	4	Alaska	20.8
	5	Arizona	6	5	Louisiana	20.2
Plague						
	1	New Mexico	35	1	New Mexico	258.9
	2	Arizona	14	2	Arizona	40.7
	3	Colorado	11	3	Colorado	36.7
	4	California	9	4	Wyoming	26.1
	5	Utah	3	5	Utah	19.2
Tularemia[c]						
	1	Arkansas	211	1	S. Dakota	1,268.0
	2	Missouri	158	2	Arkansas	1,061.5
	3	S. Dakota	74	3	Montana	531.4
	4	Oklahoma	62	4	Missouri	371.3
	5	Montana	37	5	Oklahoma	236.7

[a]Reports from 50 U.S. states, Washington, D.C., and New York City (NYC).
[b]Includes wound and unspecified botulism.
[c]Not nationally notifiable 1995–1998.

these agents after animal vaccination was implemented. Most outbreaks of foodborne botulism in the United States, especially in Alaska, have been associated with home-prepared foods, including fermented fish (22–25). High cholera incidence rates in western states and among Asians or Pacific Islanders have previously been associated with travel to cholera-endemic areas of the world (26,27). The marked seasonal distribution of cholera in the winter season resulted from a large outbreak associated with exposure on a commercial airline flight in February 1992 (27). Plague and tularemia are zoonotic diseases with recognized geographic and temporal distributions similar to those of the human cases reported to NNDSS (28–33). These patterns are probably associated with the distribution of wild rodents or domestic mammal reservoirs and hosts in the western United States or arthropod vector activity in the central states during the summer months.

Given historical trends of studied conditions, disease-specific formulas derived from the surveillance data can be used to estimate the probability that a given series of N cases of the disease would have the distribution of age, race, sex, ethnicity, and seasonal occurrence that was observed. The probability of disease occurrence estimated in this analysis was based on the assumption that these studied variables are independent. In fact, sequentially reported cases would likely cluster temporally. Therefore, the season-specific probability used in the formula to estimate the likelihood of disease cluster may be underestimated. In most cases, the probability derived from these surveillance data gives us the information on expected probability of endemic disease occurrence. Therefore, while further evaluation is needed, this information may be

Table 4. Reported cases of conditions caused by critical biologic agents, by geographic region of residence, National Notifiable Disease Surveillance System, United States, 1992-1999

	Botulism					
	Foodborne	Other	Brucellosis	Cholera	Plague	Tularemia
Geographic region[a]	Cases (%)	Cases (%)	Cases (%)	Cases (%)	Cases (%)	Cases (%)
New England	1 (0.5)	1 (0.7)	9 (1.1)	7 (3.1)	0 (0.0)	11 (1.2)
Middle Atlantic	9 (4.0)	4 (2.7)	20 (2.5)	16 (7.2)	0 (0.0)	17 (1.9)
East North Central	4 (1.8)	1 (0.7)	82 (10.1)	12 (5.4)	0 (0.0)	46 (5.2)
West North Central	2 (0.9)	1 (0.7)	37 (4.6)	3 (1.4)	0 (0.0)	296 (33.5)
South Atlantic	14 (6.3)	4 (2.7)	116 (14.3)	14 (6.3)	0 (0.0)	32 (3.6)
East South Central	10 (4.5)	2 (1.4)	20 (2.5)	0 (0.0)	0 (0.0)	24 (2.7)
West South Central	28 (12.6)	0 (0.0)	224 (27.6)	21 (9.4)	1 (1.3)	283 (32.0)
Mountain	21 (9.4)	5 (3.4)	65 (8.0)	28 (12.6)	66 (85.7)	114 (12.9)
Pacific	134 (60.1)	130 (87.8)	240 (29.5)	122 (54.7)	10 (13.0)	62 (7.0)
Total	223 (100.0)	148 (100.0)	813 (100.0)	223 (100.0)	77 (100.0)	885 (100.0)

[a]New England includes Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, and Connecticut; Middle Atlantic includes New York, New York City, New Jersey, and Pennsylvania; East North Central includes Ohio, Indiana, Illinois, Michigan, and Wisconsin; West North Central includes Minnesota, Iowa, Missouri, North Dakota, South Dakota, Nebraska, and Kansas; South Atlantic includes Delaware, Maryland, District of Columbia, Virginia, West Virginia, North Carolina, South Carolina, Georgia, and Florida; East South Central includes Kentucky, Tennessee, Alabama, and Mississippi; West South Central includes Arkansas, Louisiana, Oklahoma, and Texas; Mountain includes Montana, Idaho, Wyoming, Colorado, New Mexico, Arizona, Utah, and Nevada; Pacific includes Washington, Oregon, California, Alaska, and Hawaii.

used to compare with current disease incidence data and may serve to set reasonable thresholds for use by health departments considering initiating an epidemiologic investigation of a suspected outbreak or incident case report.

The list of critical biologic agents also includes agents that could be spread through contaminated food or water (e.g., *Salmonella* spp. or *Shigella* spp.). Because diseases caused by these food- and waterborne agents are more common in the United States compared to bioterrorism-associated diseases such as plague or tularemia, outbreaks associated with these more common agents will most likely continue to be identified through ongoing surveillance and health communication efforts that require a strong public health infrastructure. With the increasing availability of electronic health outcome data, CDC and certain states are evaluating the application of statistical aberration detection algorithms to state and national notifiable disease incidence data to aid the rapid identification of unusual disease incidence patterns (34). To support early detection of potential bioterrorist events, these or similar methods have also been applied at the state and local public health system level, where data are more timely (compared with national NNDSS data).

Even at the local and state level, however, passive notifiable disease reporting from healthcare providers and laboratories is often not timely or complete (35,36). Disease incidence reported in this analysis is likely an underestimate because of underreporting by physicians and healthcare providers. The recent terrorism-associated anthrax attacks highlighted the need for healthcare provider recognition of the syndromes associated with potential bioterrorist agents and rapid communication of relevant health outcome information between the healthcare community and the public health system. Physician case reporting is generally more complete for conditions that cause severe clinical illnesses (e.g., plague) but less complete for diseases that cause mild clinical illness (37). In the United States, the completeness of notifiable disease reporting has been estimated to range from 9% to 99% (37–40). Healthcare providers lack awareness of reporting requirements, and changes in surveillance case definitions may also lead to underreporting of notifiable diseases (37). In addition, state- and disease-specific differences in surveillance practices or in the amount of resources applied to surveillance efforts affect how actively cases are solicited or identified. Increasing awareness among healthcare providers and laboratories regarding accurate and rapid identification of conditions related to critical agents and local reporting requirements and methods is necessary to establish and maintain communication between the medical and public health communities. Increased resources (both human and technical) for surveillance at the state and local level may augment disease reporting as well.

Although most diseases caused by critical biologic agents are nationally notifiable conditions, diseases have historically been added to or deleted from the nationally notifiable disease list on the basis of criteria that did not include their etiologic agent's potential use in a bioterrorist event. Therefore, not all conditions caused by critical biologic agents are nationally notifiable diseases. For example, tularemia was temporarily removed from the nationally notifiable disease list in 1995 because of decreasing incidence. Eradicated diseases (e.g., smallpox [41–43]) are not technically nationally notifiable, nor are emerging infections (e.g., Nipah virus infection and the viral hemorrhagic fevers). However, local and state public health code typically supports the reporting of unusual events that pose a public health threat. Even when nation-

ally notifiable, however, not all conditions caused by critical biologic agents are designated as reportable in all states because states determine which conditions should be reportable in their state based on their own public health priorities and needs. Among the diseases examined in this study, only foodborne botulism was reportable in all states for the entire study period. To enhance and expand surveillance for potential bioterrorist events, CDC and the Council of State and Territorial Epidemiologists have recently added Q fever and reinstated tularemia to the list of nationally notifiable diseases. CDC continues to collaborate with the Council of State and Territorial Epidemiologists and state health departments to ensure that all nationally notifiable diseases caused by critical biologic agents are reportable in all states.

Caution should be exercised in interpreting specific incidence rates. Incidence rates for study diseases may also be underestimated because they were calculated on the basis of the U.S. population of all 50 states for the mid-study year of 1995, not limited to the population of reporting states for each year. Although rates might be underestimated, the patterns identified would not likely be affected. Although CDC and the Council of State and Territorial Epidemiologists have defined the standard case definitions for all nationally notifiable diseases, differences exist regarding how states interpret and apply these criteria. For example, although observed incidence rates of foodborne botulism were very high among children aged <1 year, these cases might be infant botulism reported as foodborne botulism. Therefore, standardized application of surveillance case definitions needs to be encouraged. Race and ethnicity information is incomplete in NNDSS data, potentially leading to underestimation of race- and ethnicity-specific incidence rates (44,45).

Overall, the incidence of conditions caused by critical microbiologic agents with the potential for use in acts of terrorism is low in the United States, as reported to NNDSS. Therefore, each case report should initially be considered a sentinel event requiring further investigation, especially reports from nonendemic regions of conditions with identified geographic distribution patterns. For potential bioterrorism-related conditions that are endemic and have low incidence, nontraditional surveillance methods (e.g., sentinel emergency department surveillance [46]) and complementary data sources (e.g., electronic laboratory reporting [47]) might be used to complement traditional sources of surveillance data (e.g., NNDSS) and can enhance our ability to detect changes in disease incidence.

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Global Illness and Deaths Caused by Rotavirus Disease in Children

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To estimate the global illness and deaths caused by rotavirus disease, we reviewed studies published from 1986 to 2000 on deaths caused by diarrhea and on rotavirus infections in children. We assessed rotavirus-associated illness in three clinical settings (mild cases requiring home care alone, moderate cases requiring a clinic visit, and severe cases requiring hospitalization) and death rates in countries in different World Bank income groups. Each year, rotavirus causes approximately 111 million episodes of gastroenteritis requiring only home care, 25 million clinic visits, 2 million hospitalizations, and 352,000–592,000 deaths (median, 440,000 deaths) in children <5 years of age. By age 5, nearly every child will have an episode of rotavirus gastroenteritis, 1 in 5 will visit a clinic, 1 in 65 will be hospitalized, and approximately 1 in 293 will die. Children in the poorest countries account for 82% of rotavirus deaths. The tremendous incidence of rotavirus disease underscores the urgent need for interventions, such as vaccines, particularly to prevent childhood deaths in developing nations.

In 1985, de Zoysa and Feachem published their landmark review of the global prevalence of rotavirus disease (1). Their analyses indicated that rotavirus accounted for 6% of diarrhea episodes and 20% of deaths caused by diarrhea in children <5 years of age in developing countries. The incidence of rotavirus disease was observed to be similar in both industrialized and developing countries, suggesting that adequate control may not be achieved by improvements in water supply, hygiene, and sanitation. Consequently, the development, trial, and widespread use of rotavirus vaccines were recommended to prevent severe and fatal rotavirus disease.

Since then, rapid progress has been made in developing and testing several rotavirus vaccine candidates (2,3). In August 1998, a live, attenuated rotavirus vaccine (Rotashield, Wyeth Laboratories, Marietta, PA) was licensed in the United States and recommended for routine immunization of U.S. infants. However, 9 months later, the use of Rotashield was suspended because reports suggested a possible association with intussusception (4). After this association was confirmed, the recommendation for use of Rotashield was withdrawn and the manufacturer stopped vaccine production.

Efforts are ongoing to develop other rotavirus vaccines, and several candidates are undergoing clinical testing (3). In addition to their safety and efficacy, the decision to implement these new rotavirus vaccines will be based on considerations of risk-benefit and cost-effectiveness. Updated estimates of rotavirus disease prevalence are a prerequisite to formulating such policy and carrying out economic analyses as well as advocacy for the next generation of rotavirus vaccines. Furthermore, each country that considers using a rotavirus vaccine may want to review the prevalence of rotavirus disease in their setting.

Since 1985, deaths from diarrheal diseases in children have declined substantially around the world, and a recent analysis suggested that deaths from rotavirus infections might also have been reduced during this period (5,6). Furthermore, scant information is available on the global extent of illness from rotavirus disease, particularly hospitalizations, which constitute a major component of total rotavirus health costs in industrialized nations. To provide updated estimates of the global illness and death from rotavirus disease in children, we reviewed studies of childhood deaths from diarrhea and of rotavirus infections published from 1986 to 2000. We also present preliminary estimates of country-specific mortality rates from rotavirus disease as targets for further study and refinement through local definition of problems. These findings should help policy makers assess the magnitude of the problem of rotavirus disease in their own countries and set priorities for interventions to prevent this disease.

Methods

Selection of Studies

The studies selected for this analysis were identified from a computer search of the scientific literature published in English between 1986 and 2000. To find studies of childhood deaths from diarrhea, we conducted a search using the keywords "childhood mortality," "deaths," and "diarrhea." We added references by reviewing the citations in these articles and by consulting with experts in the field. Because most studies of diarrhea deaths were conducted in countries with a low-income population, we supplemented

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these studies with published reports of vital registration data to analyze child death patterns in selected countries with middle- and high-income populations.

To identify studies of rotavirus disease, we conducted a search using the keyword "rotavirus" and cross-linked the articles with a second set of articles obtained from a secondary search using these keywords: incidence, prevalence, public health, death rate, mortality, surveillance, burden, suffering, distribution, area, location, and country. We also searched for permutations of these root words: epidemiol, monitor, and geograph. We then reviewed the resulting linked set of articles and narrowed it down to articles with content that was relevant to the goals of this study. We identified additional citations from references in these articles. Studies of rotavirus were included if they continued for at least 1 year, contained data on children <5 years of age, and reported using an enzyme immunoassay (EIA) or similar reliable assay to detect rotavirus. A listing of the studies included in the analyses is available in Appendix A (online only; available from: URL: http://www.cdc.gov/ncidod/EID/vol9no5/02-0562.appA.htm).

Analysis of Data

Rotavirus-Associated Illness

To estimate the extent of illness from rotavirus in children in developing countries, we first multiplied the total population of infants (0–11 months) and children (12–59 months) in those countries by the estimated annual incidence of diarrhea in the respective age groups (5,7). On the basis of published estimates from a study in Chile (8), we then distributed these diarrhea episodes into three settings: mild cases only requiring care at home; moderate cases requiring care in an outpatient clinic; and severe cases requiring hospitalization. Next, on the basis of studies we reviewed, we calculated the median proportion of diarrhea episodes attributable to rotavirus in each of the three settings. Finally, we multiplied the total number of diarrhea episodes in each setting by the estimated proportion attributable to rotavirus to yield the number of rotavirus cases in each setting.

To estimate the number of hospitalizations for rotavirus among children in industrialized countries, we multiplied estimates of the total population of children <5 years of age with rotavirus-associated hospitalization rates derived from published studies. To calculate clinic visits and episodes of rotavirus disease, we evaluated studies documenting the frequency of these outcomes relative to hospitalizations and multiplied the calculated total number of rotavirus-associated hospitalizations by corresponding factors. The figures thus obtained were combined with estimates of rotavirus illness in children in developing countries to yield the global extent of illness from rotavirus disease.

Rotavirus-Associated Deaths

To estimate the total number of child deaths from diarrhea, we plotted (for each country with available data) the fraction of deaths of children <5 years of age attributable to diarrhea against per capita gross national product (GNP). Countries were classified on the basis of GNP per capita into World Bank Income Groups (low [<U.S.$756], low-middle [U.S.$756–$2,995], high-middle [U.S. $2,996–$9,265], high [>U.S. $9,265]) (9). For each income group, we calculated the median proportion of deaths of children <5 years of age attributable to diarrhea. We then multiplied the median proportion for each income group by the total number of deaths of children <5 years of age for each country in that income group to yield country-specific estimates of the mortality rate from diarrhea. These country-specific estimates were added to calculate the global mortality rate from diarrhea.

To estimate the fraction of diarrhea deaths attributable to rotavirus, we plotted the proportion of rotavirus infection detected in children hospitalized for diarrhea that was, by virtue of the need for hospitalization, presumed to be severe. These figures were again plotted against per capita GNP for each country to yield median rotavirus detection rates for countries in the four World Bank income groups. Previously estimated diarrhea mortality rates for each country in an income group was multiplied by the median rotavirus detection rate for that income group to yield the estimated number of rotavirus deaths by country. These figures were added to yield the number of global deaths from rotavirus diarrhea. For each income strata and overall, the risk of death from rotavirus diarrhea by 5 years of age was calculated by dividing the total number of live births by the total number of deaths from rotavirus.

Results

Rotavirus Disease in Children in Developing Countries

Total Number of Diarrhea Episodes

An estimated 125 million infants 0–11 months of age and 450 million children 1–4 years of age reside in developing countries. A recent review of 27 prospective studies from 20 countries published from 1990 to 2000 estimated the incidence of diarrhea as 3.8 episodes per child per year for children ≤11 months of age and 2.1 episodes per child per year for children 1–4 years of age (5). Multiplying these age-specific incidence data with the population of children in each age group yielded an overall estimate of approximately 1.4 billion diarrhea episodes per year in children <5 years of age (Table 1). Of these, 475 million episodes are estimated to occur in ≤11-month-old infants and 945 million episodes in children 1–4 years of age.

Distribution of Diarrhea Episodes by Setting

A study from Chile demonstrated that in ≤11-month-old infants, 88.2% of diarrhea episodes required only care at home, 10.3% required a clinic visit, and 1.5% required hospitalization (8). In 1- to 4-year-old children, 91.9% of diarrhea episodes required only care at home, 7.9% required a clinic visit, and only 0.2% required hospitalization. The proportion of all diarrhea episodes requiring hospitalization was similar in another study from Thailand (10). Therefore, we applied the estimates from the Chilean study to the previously calculated total number of diarrhea episodes in each age group and distributed them into episodes requiring only home care, clinic visit, or hospitalization (Table 1). Of the total of approximately 1.4 billion diarrhea episodes in children <5 years of age, we estimated that 1.29 billion require home care only, 124 million require a clinic visit, and 9 million require hospitalization.

Number of Rotavirus Episodes in Each Setting

To estimate the number of diarrhea cases in each setting that are attributable to rotavirus, we applied proportions calculated from studies of rotavirus in children in developing countries. The review of 24 community-based studies, 13 clinic-based studies, and 72 hospital-based studies indicated that rotavirus accounted for a median of 8.1%, 18.8%, and 21.3% of diarrhea episodes in the three settings, respectively (Table 2). By multiplying these setting-specific proportions with the total number of diarrhea episodes in each setting, we calculated that rotavirus annually causes approximately 104 million episodes of diarrhea requiring home care, 23 million clinic visits, and 1.9 million hospitalizations.

Illness from Rotavirus Disease in Children in Industrialized Countries

Hospitalizations

Examination of rotavirus-specific annual hospitalization incidence from several industrialized countries demonstrated a median rate of 445 per 100,000 children (interquartile range, 283–715 per 100,000) (11–20) (Table 3). By multiplying these incidence estimates with the total population of 50,016,000 children <5 years of age in industrialized nations, we estimated that a total of 223,000 (range 142,000–358,000) rotavirus-associated hospitalizations occur in children in industrialized nations.

Clinic Visits

No reliable estimates of rotavirus-associated clinic visit rates are available for children in industrialized countries. However, studies have shown that for each child hospitalized with rotavirus diarrhea, approximately 5–10 children require a visit to a healthcare facility or physician's office (17,21,22). Therefore, we multiplied the estimated 223,000 rotavirus hospitalizations by a factor of 8 (range 5–10) to obtain an estimated total of approximately 1,781,000 (range 708,000–3,576,000) clinic visits for rotavirus disease in children <5 years of age.

Episodes Requiring Only Home Care

Studies have estimated that for each child requiring medical attention for rotavirus disease, an additional three to five children develop symptomatic disease requiring only home-care (21,22). Therefore, we multiplied the estimated 1,781,000 clinic visits by a factor of 4 (range 3–5) to estimate a total number of 7,122,000 (range 2,123,000–17,881,000) episodes of rotavirus gastroenteritis requiring only home care in children <5 years of age.

Overall Illness from Rotavirus Gastroenteritis Worldwide

By adding the total prevalence of rotavirus illness in children in developing and industrialized nations, we estimated that each year rotavirus causes approximately 111 million episodes of gastroenteritis that require home care only, 25 million clinic visits, and 2 million hospitalizations in children <5 years of age worldwide (Table 4).

Deaths from Rotavirus Disease in Children <5 Years of Age Worldwide

The proportion of deaths in children ≤5 years of age attributable to diarrhea demonstrated a declining trend

Table 1. Estimates of the annual number of diarrhea episodes among children <5 years of age in developing countries, by age group and setting[a]

	Age group		
	≤11 mo	1–4 y	Total (≤4 y)
Total population (x1,000)	125,000	450,000	575,000
No. of diarrhea episodes per child per y[b]	3.8	2.1	NA
Total diarrhea episodes (x1,000)	475,000	945,000	1,420,000
No. of episodes at home (x1,000)	418,950 (88.2)	868,455 (91.9)	1,287,405
No. of episodes in outpatients (x1,000)	48,925 (10.3)	74,655 (7.9)	123,580
No. of case-patients hospitalized (x1,000)	7,125 (1.5)	1,890 (0.2)	9,015

[a]Figures in parenthesis are percentages of total diarrhea episodes (7).
[b]From reference (5).

Table 2. Estimates of the annual number of episodes of rotavirus diarrhea among children <5 years of age in developing countries, by setting

	Home	Outpatient	Inpatient
Annual no. of diarrhea episodes (x1,000)	1,287,405	123,580	9,015
Median % of episodes with rotavirus (IQR)[a]	8.1 (4.0–12.2)	18.8 (15.0–22.0)	21.3 (17.2–28.8)
Total rotavirus episodes (range) (x1,000)	104,280 (51,496–157,063)	23,233 (18,537–27,188)	1,920 (1,551–2,596)

[a]IQR, interquartile range.

with increasing income level (Figure 1A); the median proportion for low-income countries was 21%; for low-middle income countries, 17%; for high-middle income countries, 9%; and for high-income countries, 1%. We multiplied these income stratum-specific median estimates with the combined ≤5 mortality estimates for countries in each of the four income strata to yield an overall estimate of 2.1 million (range 1.7 million–3.0 million) diarrhea deaths per year (Table 5). Of the median 2.1 million diarrhea deaths, 85% (N=1,805,000) occurred in children from low-income countries.

The proportion of diarrhea hospitalizations attributable to rotavirus demonstrated an increasing trend with increasing income level (Figure 1B); the median for low-income countries was 20%; for low-middle income countries, 25%; for high-middle income countries, 31%; and for high-income countries, 34%. We multiplied these stratum-specific proportions with the median estimate of total diarrhea deaths for countries in each of the four income strata to yield an estimated 352,000–592,000 (median 440,000 deaths) per year from rotavirus. Of the median 440,000 deaths, 82% (N=361,000) occurred in children from low-income countries.

To obtain country-specific estimates of deaths from diarrhea and rotavirus disease, we first multiplied United Nations Children's Fund estimates of total number of deaths of children <5 years of age for each country in each income stratum with the median proportion for that stratum of deaths in children <5 years of age attributable to diarrhea. The obtained country-specific estimates of diarrhea deaths were further multiplied by the median proportion for that stratum of diarrhea hospitalizations attributable to rotavirus. The results of these calculations are presented in the Appendix B (online only; available from: URL: http://www.cdc.gov/ncidod/EID/vol9no5/02-0562_appB.htm) and shown in Figure 2.

Discussion

The findings of this study demonstrate the tremendous amount of global illness and deaths caused by rotavirus disease. Each year, rotavirus causes an estimated 111 million episodes of diarrhea requiring only home care, 25 million clinic visits, 2 million hospitalizations, and 352,000–592,000 deaths (median 440,000 deaths) in children <5 years of age. In other words, by 5 years of age, almost all children will have an episode of rotavirus gastroenteritis, 1 in 5 will require a clinic visit, 1 in 65 will require hospitalization, and approximately 1 in 293 will die (Figure 3). The incidence of rotavirus disease is similar in children in both developed and developing nations. However, children in developing nations die more frequently, possibly because of several factors, including

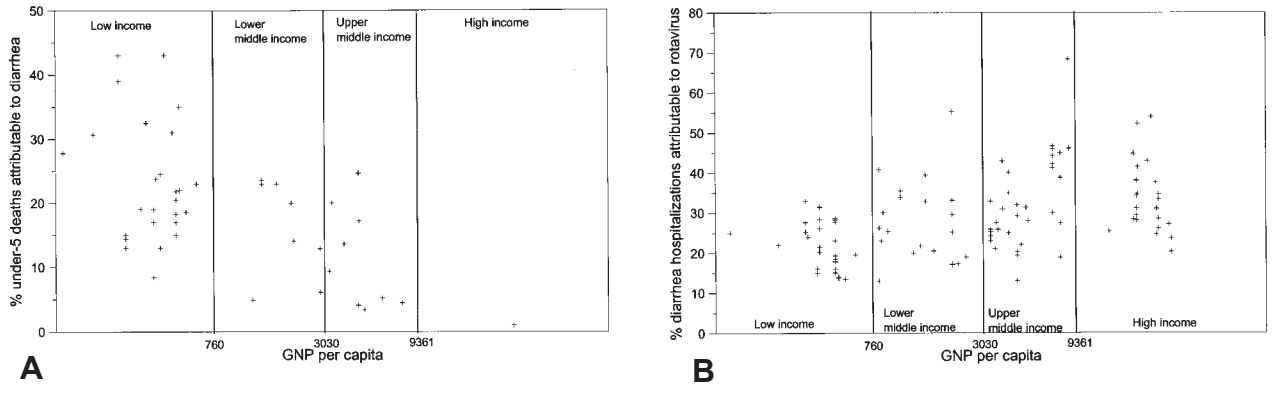
Table 3. Annual incidence of hospitalizations for rotavirus gastroenteritis in children <5 years of age in selected industrialized countries

Country (reference)	Y	Annual incidence/100,000 children	Cumulative incidence by 5 y of age
Spain (11)	1989–1995	250	1 in 80
Netherlands (12)	1998	270	1 in 74
United States (13)	1993–1995	274	1 in 73
Poland (14)	1996	310	1 in 65
Sweden (15)	1993–1996	370[a]	1 in 54
United Kingdom (16)	1993–1994	520	1 in 38
Finland (17)	1985–1995	610	1 in 33
Australia (18)	1993–1996	750	1 in 27
Hungary (19)	1993–1996	840[a]	1 in 24
Australia (20)	1991–1993	870	1 in 23

[a]Incidence for children <4 years of age.

Table 4. Annual global illness incidence from rotavirus disease among children <5 years age, by setting

	No. (range) of episodes of rotavirus disease (x1,000)		
Setting	Developing countries	Industrialized countries	Total
Home	104,280 (51,496–157,063)	7,122 (2,123–17,881)	111,402 (53,619–174,946)
Outpatient	23,233 (18,537–27,188)	1,781 (708–3,576)	25,017 (19,245–30,764)
Inpatient	1,920 (1,551–2,596)	223 (142–358)	2,143 (1,693–2,954)



	Low income	Lower middle income	Upper middle income	High income
Median	21	17	9	1
IQR	17-30	11-23	5-17	NA

	Low income	Lower middle income	Upper middle income	High income
Median	20	25	31	34
IQR	16-27	20-33	25-42	28-38

Figure 1. A. Percentage of deaths in children <5 years that are attributable to diarrhea for countries in different World Bank income groups, by gross national product (GNP) per capita of the country. B. Percentage of diarrhea hospitalization attributable to rotavirus for countries in different World Bank income groups, by GNP per capita of the country. IQR, interquartile range.

poorer access to hydration therapy and a greater prevalence of malnutrition. An estimated 1,205 children die from rotavirus disease each day, and 82% of these deaths occur in children in the poorest countries.

In 1986, the Institute of Medicine (IOM) estimated, on the basis of published studies and field experience, that annually rotavirus causes approximately 110 million episodes of mild diarrhea, 10 million episodes of moderate to severe diarrhea, and 9 million episodes of severe diarrhea in children <5 years of age worldwide (23). Our estimate of the incidence of rotavirus gastroenteritis is similar to the IOM estimate and is consistent with a recent analysis demonstrating that overall diarrhea illness in children worldwide has not declined appreciably in the past two decades (5). However, our estimate of total hospitalizations from rotavirus disease is substantially lower than the IOM estimate. The difference might be explained, in part, by the relatively low hospitalization rate for diarrhea in the study in Chile (1.5% of all diarrhea episodes) used in our calculations (8). However, a study in a low-income urban community in Thailand showed a similar hospitalization rate (1% of all diarrhea episodes) among children with diarrhea (10), giving us added confidence in our estimates. Increased use of oral rehydration therapy and improvements in nutritional status are two factors that might explain a possible reduction in severe rotavirus cases without a concomitant decline in diarrhea incidence (24,25).

Our estimate of 352,000–592,000 deaths (median: 440,000 deaths) from rotavirus disease each year is similar to a recent estimate of 418,000–520,000 deaths proposed by Miller and McCann (6) but is substantially lower than the 1985 IOM estimate of 873,000 deaths. This decline in the rotavirus mortality rate parallels the decline in overall deaths from diarrhea in children in the past two decades, from an estimated 4.6 million deaths in 1982 (26) to our estimate of 2.1 million deaths in 2000. However, the patterns of diarrhea deaths reported in this study reflect the situation a decade ago, when most studies that we reviewed were conducted. Analyses of vital registration data from several countries have suggested that the proportion of deaths from diarrhea may have declined further in recent years (27). Other studies have noted marked discrepancies in the analysis of cause of death from vital registration data and prospective observational studies (28). Careful and detailed analyses are required to assess the current magnitude of the deaths from diarrhea in children,

Table 5. Global estimates of the annual number of diarrhea and rotavirus deaths among children <5 years of age, by income group

Income group	Total no. (x1,000)		Diarrhea deaths		Rotavirus deaths[b]		Risk of dying from rotavirus by age 5
	Births	Deaths	Median % (IQR[a]) of total deaths	Median no. (IQR) of deaths (x1,000)	Median % (IQR) of diarrhea hospitalizations	Median no. (IQR) of deaths (x1,000)	
Low	70,447	8,595	21 (17–30)	1,805 (1,461–2,579)	20 (16–27)	361 (289–487)	1 in 205
Low middle	37,402	1,609	17 (11–23)	274 (177–370)	25 (20–33)	69 (55–90)	1 in 542
Upper middle	11,520	366	9 (5–17)	33 (18–62)	31 (25–42)	10 (8–14)	1 in 1,152
High	9,931	60	1	<1	34 (28–38)	<1	1 in 48,680
Total	129,300	10,630	NA	2,112 (1,657–3,012)	NA	440 (352–592)	1 in 293

[a]IQR, interquartile range; NA, not applicable.
[b]The estimated number and range of deaths from rotavirus are derived by multiplying the median and IQR of diarrhea hospitalizations attributable to rotavirus by the median number of deaths caused by diarrhea for each stratum

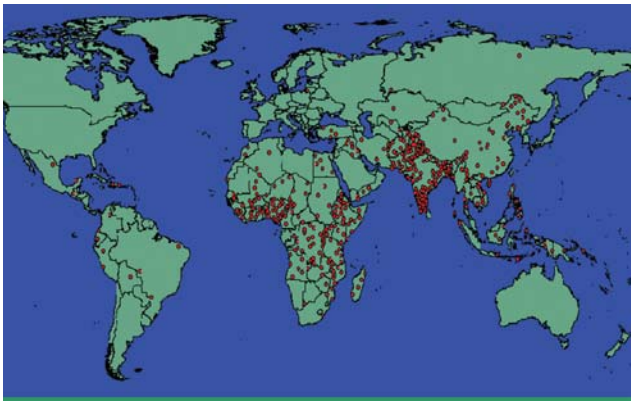


Figure 2. Estimated global distribution of 440,000 annual deaths in children caused by rotavirus diarrhea. One dot=1,000 deaths

and the results will directly affect our estimates of deaths from rotavirus disease. For example, if our estimated proportion of severe diarrhea cases attributable to rotavirus is applied to the recent estimate of 2.5 million annual diarrhea deaths developed by Kosek et al. (5), we estimate 416,000–700,000 annual deaths (median:520,000 deaths) from rotavirus disease.

Another important factor that could affect our estimate of rotavirus deaths is the possibility that as the overall mortality rate from diarrhea has declined over the past two decades, the proportion of diarrhea deaths attributable to rotavirus may have increased, given that this pathogen is often transmitted from person to person and is difficult to control through improvements in hygiene and sanitation. This hypothesis is supported by data from Mexico, demonstrating that whereas deaths from diarrhea declined substantially from 1989 to 1995, the decline was less evident for winter seasonal deaths in children <2 years of age whose illness met the epidemiologic features of rotavirus diarrhea (29). In addition, some recent studies of rotavirus based on hospital surveillance in developing countries have demonstrated detection rates in excess of 50% (30,31). If this trend is confirmed as additional data become available from ongoing surveillance studies in several regions of the world, the estimates of rotavirus deaths reported in this article will have to be revised to reflect current mortality patterns.

This review, based on a compilation of studies varying in design, time, and place, has several inherent limitations that we attempted to address. Because of the marked seasonality of rotavirus disease and the variation in the sensitivity and specificity of diagnostic tests for rotavirus, we restricted this review to studies that lasted at least 1 year and used reliable assays for the detection of rotavirus. To account for known temporal changes in the magnitude and patterns of diarrhea-associated childhood deaths, we reviewed only studies published within the last 15 years and used the most recent available estimates of total deaths in children <5 years to calculate estimates of diarrhea deaths. Furthermore, because regional boundaries are primarily based on geographic and political considerations and do not necessarily reflect important determinants of health, we used indicators of socioeconomic status to stratify our analyses of mortality patterns.

Nevertheless, we could not adequately account for several factors that may have affected our findings. First, the studies we reviewed were conducted in selective populations that may not have been representative of the entire country. Second, most diarrhea mortality studies used verbal autopsies to determine the cause of death, which may affect our estimates because these methods have variable sensitivity and specificity and it is difficult, if not impossible, to assign a single cause of death for children who died with multiple conditions (32–34). Finally, because of a time lag between the conduct of studies and publication of their findings, our data likely do not reflect the most current trends of diarrhea and rotavirus disease patterns.

In 1998, the first rotavirus vaccine was licensed in the United States, offering an encouraging opportunity for the prevention of this disease. However, the vaccine was withdrawn within a year of licensure because it caused an estimated one case of intussusception for every 12,000 vaccinated infants. The lack of sufficient data on the efficacy of vaccine in developing countries as well as political and ethical considerations diminished prospects for its use in these settings. Our findings demonstrate that the next generation of rotavirus vaccines will have greatest impact in developing countries where the disease burden is greatest. Our estimates of rotavirus mortality rates for individual countries, although developed with relatively crude methods, compare favorably with those from more detailed analysis conducted in selected countries. For example, good concordance was noted between the previous figures and our estimates of rotavirus mortality for Bangladesh (14,850–27,000 vs. 13,104 deaths) (35), Peru (1,600 vs. 1,360 deaths) (36), and India (98,000 vs. 100,800 deaths) (37). The establishment of regional networks for rotavirus surveillance in sentinel hospitals will facilitate more time-

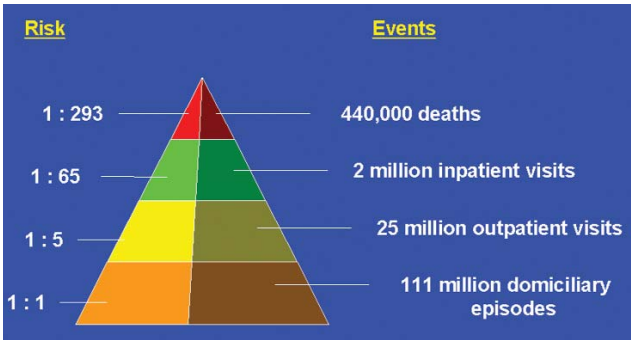


Figure 3. Estimated global prevalence of rotavirus disease.

ly and refined estimates of disease illness and death. These data, along with information on illness and costs of rotavirus infections, will assist policy makers in assessing the magnitude of the problem of rotavirus in their own setting and in setting priorities for interventions, such as the next generation of rotavirus vaccines, which may be available in the near future.

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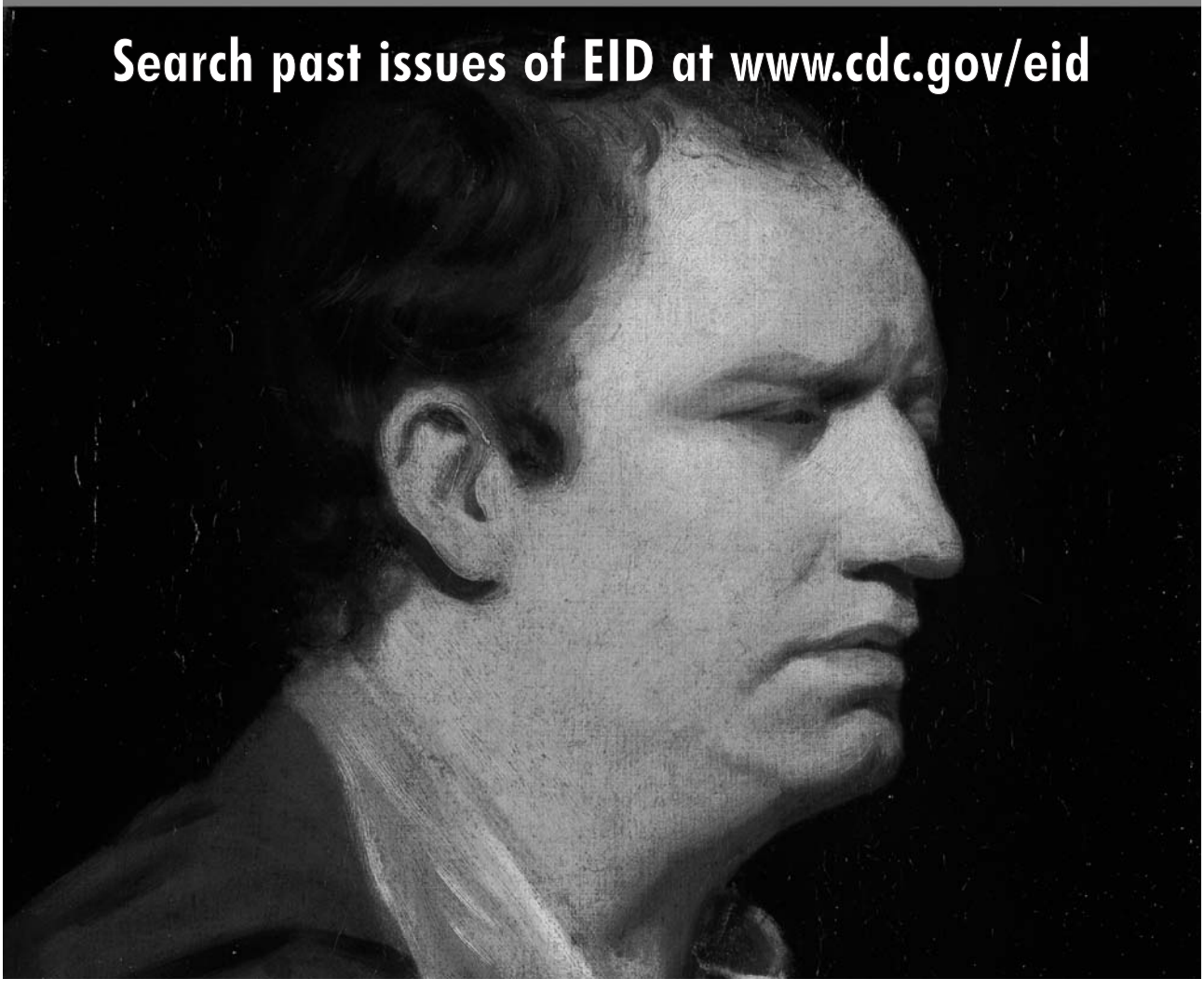

Seasonal Patterns of Invasive Pneumococcal Disease

Scott F. Dowell,*† Cynthia G. Whitney,† Carolyn Wright,† Charles E. Rose, Jr.,† and Anne Schuchat†

Pneumococcal infections increase each winter, a phenomenon that has not been well explained. We conducted population-based active surveillance for all cases of invasive pneumococcal disease in seven states; plotted annualized weekly rates by geographic location, age, and latitude; and assessed correlations by time-series analysis. In all geographic areas, invasive pneumococcal disease exhibited a distinct winter seasonality, including an increase among children in the fall preceding that for adults and a sharp spike in incidence among adults each year between December 24 and January 7. Pneumococcal disease correlated inversely with temperature (r –0.82 with a 1-week lag; p<0.0001), but paradoxically the coldest states had the lowest rates, and no threshold temperature could be identified. The pattern of disease correlated directly with the sinusoidal variations in photoperiod (r +0.85 with a 5-week lag; p<0.0001). Seemingly unrelated seasonal phenomena were also somewhat correlated. The reproducible seasonal patterns in varied geographic locations are consistent with the hypothesis that nationwide seasonal changes such as photoperiod-dependent variation in host susceptibility may underlie pneumococcal seasonality, but caution is indicated in assigning causality as a result of such correlations.

As with many infectious diseases, the incidence of invasive pneumococcal infection rises and falls in an annual seasonal pattern that has been repeatedly documented but never well explained. The winter increase in cases has been attributed to cold weather, lower humidity, the crowding together of susceptible hosts, associated viral infections, and air pollution (1–3). Explanations for the cause of pneumococcal seasonality often appear contradictory. A temporary increase in the incidence of invasive disease was associated with two severe winters in the Netherlands (4), but in Alaska, the number of invasive cases is highest in summer months (5). The seasonal variation has been more apparent in adult invasive pneumococcal disease than in pediatric cases (1), although some seasonal variation in acquisition of nasopharyngeal colonization also has been documented in children (6). These disparate observations have been difficult to reconcile with a unifying explanation for pneumococcal seasonality.

A recent hypothesis proposes that the seasonal variation in the incidence of some infectious diseases is attributable to seasonal variation in host physiology, such as the density of a cellular receptor or the activity of the immune response (7). Many mammals exhibit distinct physiologic changes with the changing seasons, typically timed to the light-dark cycle and mediated by melatonin (humans exhibit some such changes) (8–11). Experiments with mice provide some evidence that susceptibility to fatal pneumococcal disease varies with the animal's innate circadian rhythm (12–14). Determining the relevance, if any, of these observations to human pneumococcal infections would be advanced by a detailed description of the seasonal patterns of pneumococcal infections of various age groups and different geographic areas with distinct weather patterns. We evaluated data from a population-based surveillance system in seven geographic areas in the United States (Active Bacterial Core Surveillance) to describe the seasonal variation in invasive pneumococcal disease and explore these hypotheses.

Methods

Surveillance for invasive pneumococcal disease was performed from January 1, 1996, through December 31, 1998, in the counties of seven U.S. states, as consistently defined in the Active Bacterial Core Surveillance of the Emerging Infections Program Network. Geographic location was grouped by state and by latitude (Figure 1), including eight counties in Georgia and five counties in Tennessee (latitude 33°–35° north; defined here as southern sites), one county in California and six counties in Maryland (latitude 37°–39° north; middle sites), and the entire state of Connecticut, seven counties in Minnesota, and three counties in Oregon (latitude 41°–45° north; northern sites). Invasive disease caused by group A streptococci and group B streptococci was also assessed for comparison with pneumococcal disease.

Invasive disease was defined as disease in which an organism had been isolated from a normally sterile site (such as blood or cerebrospinal fluid) in a resident of the surveillance area. Each case was considered to have

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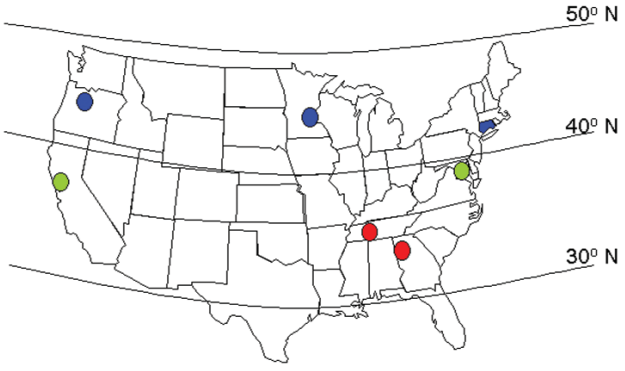

Figure 1. Map of the continental United States showing the approximate locations of the seven surveillance sites, grouped for some analyses as southern sites (illustrated in red), middle sites (green), and northern sites (blue).

occurred on the date the culture was obtained. In each surveillance area, project personnel communicated regularly with contacts in all microbiology laboratories serving acute-care hospitals and completed standardized case-report forms. Audits were performed at least every 6 months to evaluate reporting sensitivity and identify unreported cases. All isolates were sent to the Centers for Disease Control and Prevention or the University of Texas Health Science Center at San Antonio on blood agar slants and confirmed as pneumococci on the basis of optochin susceptibility and bile solubility.

Daily surface weather data were obtained from weather stations in each surveillance site (Atlanta, Memphis, San Francisco, Baltimore, Hartford, Minneapolis, and Portland) from the National Climate Disease Center, Asheville, North Carolina (available from: URL: http://www.ncdc.noaa.gov). Sunrise and sunset times for each surveillance week were obtained for each of the above seven sites from the U.S. Naval Observatory, Washington, D.C. (available from: URL: http:// aa.usno.navy.mil). Monthly figures for electric utility gas consumption were obtained from the Energy Information Administration, Washington, D.C. (available from: URL: http://www.eia. doe.gov), and figures for public construction expenditures were obtained from the U.S. Census Bureau, Washington, D.C. (available from: URL: http://www.census.gov).

Weekly numbers of cases were converted to annualized rates by dividing by the census population in the relevant counties for the appropriate surveillance year and multiplying by 100,000 population and 52 weeks (to get cases per 100,000 population per year). We then plotted annualized rates by state, latitude group (as categorized above), and age (adults ≥18 years and children).

Time series analysis was performed with SAS software (version 8.2, SAS Institute, Inc., Cary, NC) and Epi Info software version 6.02 (Centers for Disease Control and Prevention, Atlanta, GA). Annualized weekly rate correlations with mean weekly temperature (in degrees F), total weekly precipitation (100ths of inches), and mean weekly minutes of darkness were calculated as Pearson correlation coefficients with time lags ranging from 0 to 8 weeks. Time lags were limited to an 8-week range because the highest correlations from exploratory analysis in the annualized weekly rate with the climatic data occurred within 2 months. Correlations with monthly gas consumption and construction expenditures were explored with time lags of 20 to 36 months because the data were available from 1999 to 2001, whereas the weekly pneumococcal disease rates were available from 1996 to 1998. Weather data were missing from Atlanta for 1996 and were not included; occasional missing readings were otherwise replaced by interpolation.

Results

Over the 3-year period, 11,614 cases of invasive pneumococcal disease were identified among the approximately 15,221,605 residents of the surveillance areas. The annualized weekly rate of invasive pneumococcal disease was distinctly seasonal, varying from approximately 10 cases per 100,000 population during the summer to approximately 35 cases per 100,000 during the winter (Figure 2). No seasonal variation was seen in the incidence of group B streptococcal infection, and a mild spring peak was seen in group A streptococcal infection. A prominent spike in the rate of invasive pneumococcal disease to 50–75 cases per 100,000 occurred during the last week of December and the first week of January in each of the surveillance years.

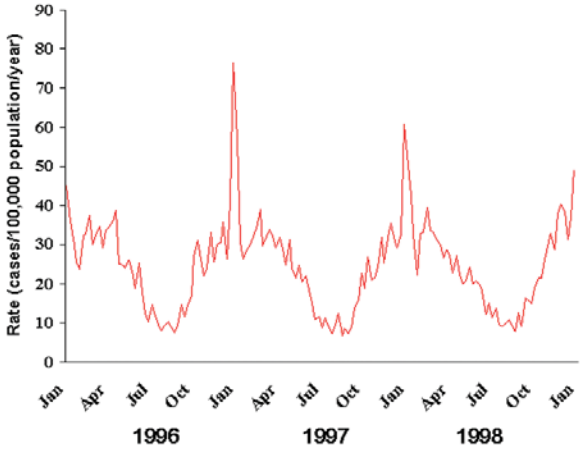

Figure 2. Weekly rates of invasive pneumococcal disease in the United States, January 1996–December 1998. Weekly numbers of cases from active surveillance areas in California, Connecticut, Georgia, Maryland, Minnesota, Oregon, and Tennessee were divided by the population under surveillance that year and multiplied by 52 to give annualized weekly rates.

The seasonal patterns were similar when the data from each state were plotted separately and when the states were grouped into the three latitude groups (Figure 3). The number of weekly cases increased approximately fourfold from summer to winter, and a prominent spike in the number of weekly cases occurred during the last week of December and the first week of January.

The pattern of seasonal variation was somewhat different in children compared to adults (Figure 4). The annual rise in incidence among children preceded that of adults, reaching the highest incidence during September and maintaining a high incidence throughout the fall. The spike in incidence during the last week of each year and first week of the subsequent year was seen in adults but not in children.

We further explored the characteristics of persons with invasive pneumococcal disease during the prominent annual spike (defined as December 20–January 10 of each year) and those experiencing invasive disease during the surrounding weeks (December 1–19, and January 11–31). Patients with invasive disease occurring during the spike and nonspike periods had similar demographic characteristics, except that adults were disproportionately represented during the spike (Table). After children were excluded, the median age of adults with invasive disease during the spike was 60 years, compared with 56 years for adults in the surrounding weeks (p=0.05). No similar spike in incidence occurred for either group A or group B streptococcal disease.

The seasonal peaks in invasive pneumococcal disease in each of the three latitude groups correlated with increases in the number of hours of darkness and with cold temperatures in the winter, but correlation was poor with seasonal variations in total precipitation (Figure 3). Correlations were highly significant (Pearson's correlation coefficients all ≤ -0.5; $p<0.0001$) for pneumococcal disease and temperature, with time lags ranging from 0 to 8 weeks (highest r –0.82 with a 1-week lag). Correlations were also highly significant (r all ≥ 0.7; $p<0.0001$) for minutes of darkness and pneumococcal disease (highest r +0.85 with a 5-week lag). Temperature and photoperiod were themselves highly correlated with each other (r –0.95; $p<0.0001$). Precipitation did not correlate well with pneumococcal disease (r all ≤ 0.3, with best correlation of 0.29 using a 0-week lag).

The temperature curves for the individual states showed distinct patterns (data not shown), as did the temperature curves when sites were grouped by latitude (Figure 3c), but these variations were not associated with site-specific variation in pneumococcal disease. For example, the northern sites, with temperatures averaging 10°–20° lower than the southern sites throughout the year, did not have higher rates of invasive pneumococcal disease (Figure 3a). No evi-

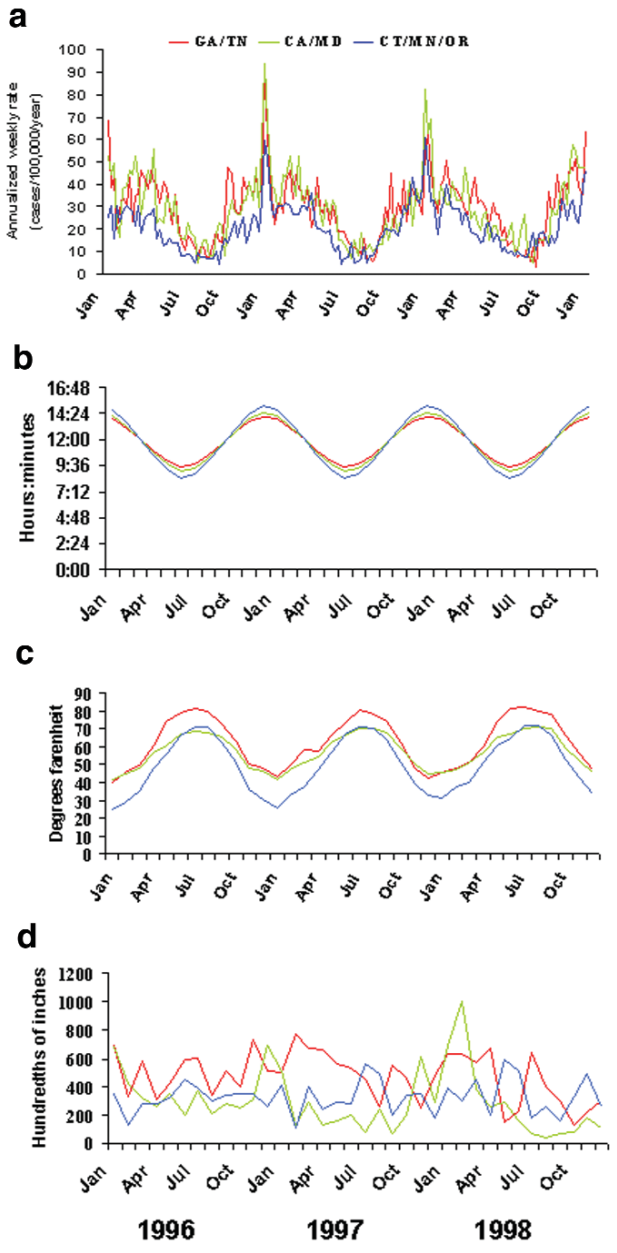

Figure 3. The relationship of invasive pneumococcal disease to photoperiod, temperature, and precipitation in three surveillance areas, grouped by latitude, 1996–1998. Southern surveillance areas were in Georgia and Tennessee (red lines; 33°–35° north latitude), middle areas were in California and Maryland (green lines; 37°–39° north latitude), and northern areas were in Connecticut, Minnesota, and Oregon (blue lines; 41°–45° north latitude). a: Rates of invasive pneumococcal disease; b: hours of darkness calculated for the 15th day of each month; c: mean monthly surface air temperature recorded at weather stations in each of the seven surveillance sites. d: total monthly precipitation recorded at weather stations in each of the seven surveillance sites. Pneumococcal disease correlated directly with photoperiod (r 0.85 with a 5-week lag; $p<0.0001$), indirectly with temperature (r –0.82 with a 1-week lag; $p<0.0001$), and poorly with precipitation (r 0 to <0.3).

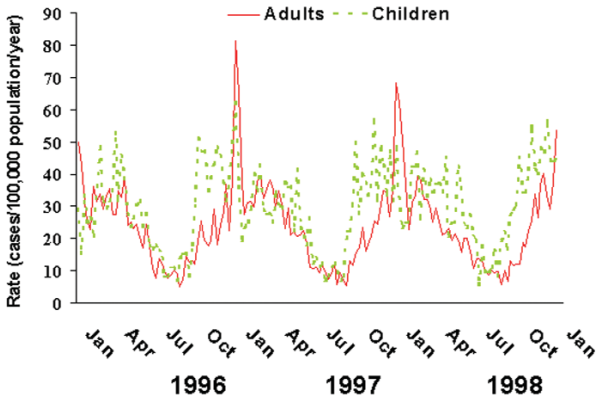

Figure 4. Weekly rates of invasive pneumococcal disease in children (dotted line; ages 0–17 years), and adults (solid line; age >18 years) in the United States, 1996–1998. Weekly numbers of cases from seven active surveillance areas were divided by the age-specific population and multiplied by 52 to give annualized weekly rates.

dence of a longer pneumococcal season in northern sites than in southern sites was evident, a finding that might have been expected if a threshold temperature existed, below which pneumococcal disease rates increase.

Seasonal variations in photoperiod, or the length of the light-dark period, also corresponded with the seasonal variation in pneumococcal disease. Photoperiod was a seasonally varying phenomenon with less site-to-site variation than temperature (Figure 3b), similar to the consistent pattern of pneumococcal disease rates across the sites. The variation in the magnitude of the photoperiod curves was slightly more pronounced for northern sites than for southern sites (Figure 3b), a pattern which was not seen in invasive pneumococcal disease (Figure 3a). Associations between the seasonal increases in invasive pneumococcal disease and seemingly unrelated phenomena were not difficult to demonstrate, such as the correlations with seasonal variation in public construction expenditures (r –0.84 with a 35-month lag) and electric utility gas consumption (r –0.92 with a 22-month lag) (Figure 5).

Discussion

A distinctive and regular seasonal variation in the incidence of invasive pneumococcal disease was confirmed by this analysis, as has been reported by others (1,3,15–17). We found that the patterns of seasonal variation were remarkably consistent across our seven geographically distinct surveillance areas, and we used the site-specific differences in weather across these areas to evaluate proposed explanations for the cause of pneumococcal seasonality. The unique seasonal patterns in children's infections as compared to those of adults, and the peculiar spike in incidence in adults during the same 1- to 2-week period each year remain incompletely explained, although these observations should provide ample opportunities for testing hypotheses in future studies.

The shapes of the invasive pneumococcal disease curves were remarkably consistent across all seven surveillance sites, including the timing of the summertime nadir, the fall upswing in incidence, and the midwinter peak. These patterns were consistent despite marked differences in rainfall patterns and, to a lesser extent, differences in air temperature across these geographically widespread sites. If colder temperature is what drives the winter increase in pneumococcal disease, we might have expected higher rates throughout the year from northern sites, but that is not what we found. Alternatively, pneumococcal disease might increase when temperatures drop below some biologic threshold. Such biologic thresholds are present in certain temperature-sensitive viruses, for example, that are useful as vaccines because they are not viable in the warmer temperatures of the human lung (18). Such a temperature threshold for pneumococcal disease might be expected to result in a longer "pneumococcal season" in northern sites, a pattern we also did not find.

Temperature and photoperiod variations need to be investigated further with datasets obtained by using similar methods from regions with wider latitude and temperature variations than we used here. Preliminary review of a smaller dataset of invasive pneumococcal disease from a different surveillance system in Alaska did not identify elevated rates of pneumococcal disease associated with the

Table. Characteristics of persons with invasive pneumococcal disease during an annual winter spike in incidence compared with those experiencing invasive pneumococcal disease during the surrounding weeks, 1996–1998[a]

	% of patients with characteristic		
Characteristic	Nonspike (N=1,647)	Spike (N=1,351)	p value[b]
Sex (% male)	54.9	51.4	0.07
Race (% white)	56.6	60.8	0.07
Age (% adult)	74.3	81.7	0.0000013
Survival	87.1	87.5	0.92
State			0.49
California	6.0	4.9	–
Connecticut	17.7	18.9	–
Georgia	25.6	25.3	–
Maryland	20.2	19.1	–
Minnesota	10.4	11.3	–
Oregon	6.8	5.8	–
Tennessee	13.3	14.7	–
Syndrome			0.24
Bacteremia	35.6	33.0	–
Pneumonia	56.4	59.6	–
Meningitis	4.9	4.6	–

[a]The spike period was defined as December 20–January 10 of each year, and the nonspike periods were December 1–19 and January 11–31.
[b]p value calculated by chi-square test of the 2 x N table, with Yates correction.

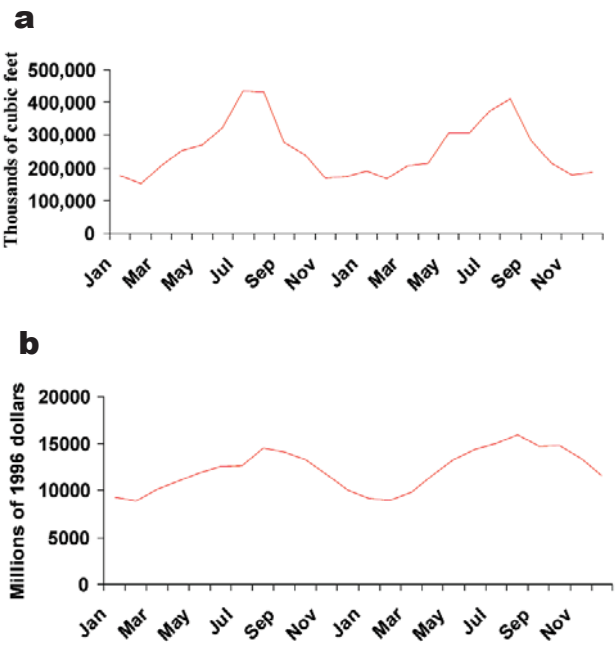

Figure 5. Seasonal variations in U.S. electric utility gas consumption (a) and public construction expenditures (b). Both parameters correlated indirectly with the invasive pneumococcal disease rates (gas consumption: r –0.92 with a 22-month lag; and construction: r –0.84 with a 35-month lag). Data were obtained from the Energy Information Administration and the U.S. Census Bureau, respectively.

colder weather and longer winter and also did not display the consistent seasonal variations seen in our continental U.S. sites (J. Butler, pers. comm.).

Some of the apparent variation in incidence of invasive pneumococcal disease seen in different studies may be attributable to variations in blood-culturing patterns (19,20). Geographic variation in blood culture practices may be more likely to influence the size of seasonal peaks and less likely to affect their timing, as we observed. Strengths of this analysis include the large population under surveillance and the consistent methods used to identify cases across all seven geographically distinct surveillance areas; these factors should tend to minimize any effects of local differences in blood-culturing practices or institutional variation in case ascertainment.

The similar seasonal patterns observed in our seven surveillance sites, despite their wide variations in air temperature, precipitation, and other weather patterns, argue that the signal guiding pneumococcal seasonal variations is one that is more consistently present in all sites. Photoperiod has been shown to be the most pervasive signal for seasonal changes in biologic systems (10,21–23), and we believe our findings are consistent with the proposal that photoperiod also contributes to the seasonality of invasive pneumococcal disease in humans. The sinusoidal variation in photoperiod across the sites correlated closely with the pattern of invasive pneumococcal disease. We did not find a greater magnitude of pneumococcal variation from northern sites, and definitively identifying photoperiod, rather than temperature, as the driving phenomenon was not possible from this dataset. Because temperature and photoperiod are themselves highly correlated, evaluating the relative contributions of each to invasive pneumococcal disease will likely require further exploration with more years of data from more geographically diverse sites.

We emphasize that correlating seasonal variations in invasive pneumococcal disease with temperature and photoperiod provides insufficient evidence to establish a causal link. Using brief searches of the Internet, we readily identified seasonal variations in housing construction, gas consumption, and other seemingly unrelated phenomena that were also significantly correlated with the seasonal variation in pneumococcal disease. Because so many phenomena vary seasonally, evaluating one or two hypothesized causes for seasonal variation in disease and concluding that the close correlation supports causality is a pitfall all investigators in this field must take care to avoid.

Accumulating evidence is supporting the biologic plausibility that preceding respiratory virus infections, influenza in particular, increase susceptibility to invasive pneumococcal disease (24,25). We did not have comparable data on virologically confirmed influenza infections from each of the seven surveillance sites to include in this analysis, but such information should be sought in future evaluations.

Some experimental evidence exists to support the concept that cyclical variation in susceptibility to pneumococcal infections is present in the mammalian host, and that the light-dark cycle provides the signal that entrains this cyclical pattern. Mice kept in windowless rooms with a controlled light-dark cycle are less susceptible to pneumococcal infection during the dark phase (12). When such mice were inoculated intraperitoneally with virulent pneumococci during the dark phase (0400), there was a slower increase in bacteremia (13), and the mice survived significantly longer, compared to those challenged during the light phase (12). In blinded mice, this pattern of decreased susceptibility was maintained on an approximately 24-hour (circadian) pattern but cycled out of phase with the room lighting. These findings indicate the presence of an endogenous rhythm of susceptibility to pneumococcal infection that is normally entrained to a strict 24-hour pattern by visual detection of the daily light-dark cycle (14). Whether this circadian variation in susceptibility is accompanied by seasonal variation has not been evaluated, although the two periodic phenomena are closely related in most biologic systems (23). Humans retain the physiologic capacity to respond to changes in photoperiod (11,

26–28), but some evidence exists that artificial lighting blunts these responses (29). Moreover, the degree to which seasonal changes in light cycles influences human physiology, if at all, is not clear.

The seasonal pattern in children was distinct from that in adults, consistent with differences in clinical disease in children and with the observations of others (1,5,30,31). Children are the reservoirs for pneumococci in the community, with nasopharyngeal colonization varying slightly (6), if at all (32), throughout the year. We observed a seasonal peak in children that was broader and flatter than that in adults, with an early autumn rise to the peak and an absence of the midwinter spike. Others have described this as a biphasic pattern, with a flattening or drop in incidence in midwinter (30,31,33,34); we did not observe this pattern. We speculate that the early autumn rise may be associated with the return to school and exchange of new serotypes, which may then be transmitted to adult contacts. The substantial variation in invasive disease despite fairly steady carriage supports a role for increasing host susceptibility or other predisposing factors, rather than appearance and disappearance of the pathogen, as an underlying explanation.

The prominent midwinter spike in incidence we observed does not appear to be an artifact of the surveillance system. The spike, present in all seven states, was not seen for group A or group B streptococcal disease, which were reported through the same system. Persons with invasive disease during the spike were significantly older but otherwise demographically similar to those with invasive disease during the surrounding weeks. The consistent timing of the spike during the weeks of December 24–January 7 is provocative. This is a time when many U.S. families gather for Christmas and New Year's holidays, perhaps providing an opportunity for exposure of older relatives to new serotypes from young children at a time when their annual susceptibility to pneumococcal disease is at its peak. Previous studies have documented an association between exposure to young children and invasive pneumococcal disease (35,36). Others have not reported such a spike, but most such analyses have grouped the data by month, an approach that would have led us to miss the spike in our data.

We encourage others with similar databases to examine their data by weekly intervals, to distinguish patterns in adults from those in children, and to report information on latitude, temperature patterns, humidity, and other variables to allow for a consistent exploration of the influences on pneumococcal seasonality. Variation in susceptibility in the human host is one hypothesis that should be routinely considered.

Dr. Dowell is the director of the International Emerging Infections Program in Thailand, the first of a planned network of such collaborations between the Centers for Disease Control and Prevention and host country ministries of health. His research interests include the seasonality of infectious diseases and the detection and control of emerging infections.

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Entamoeba moshkovskii Infections in Children in Bangladesh

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Entamoeba moshkovskii cysts are morphologically indistinguishable from those of the disease-causing species *E. histolytica* and the nonpathogenic *E. dispar*. Although sporadic cases of human infection with *E. moshkovskii* have been reported, the organism is considered primarily a free-living amoeba. No simple molecular detection tool is available for diagnosing *E. moshkovskii* infections. We used polymerase chain reaction (PCR) to detect *E. moshkovskii* directly in stool. We tested 109 stool specimens from preschool children in Bangladesh by PCR; 17 were positive for *E. histolytica* (15.6%) and 39 were positive for *E. dispar* (35.8%). In addition, we found that 23 (21.1%) were positive for *E. moshkovskii* infection, and 17 (73.9%) of these also carried *E. histolytica* or *E. dispar*. The high association of *E. moshkovskii* with *E. histolytica* and *E. dispar* may have obscured its identification in previous studies. The high prevalence found in this study suggests that humans may be a true host for this amoeba.

Entamoeba moshkovskii, considered to be primarily a free-living amoeba, is indistinguishable in its cyst and trophozoite forms from *E. histolytica* (the cause of invasive amebiasis) and *E. dispar* (a common noninvasive parasite), except in cases of invasive disease when *E. histolytica* trophozoites may contain ingested red blood cells. *E. moshkovskii* has so far rarely been shown to infect humans; however, the organism appears to be ubiquitous in anoxic sediments. Although the early isolations of this species were from sewage, *E. moshkovskii* can also be found in environments ranging from clean riverine sediments to brackish coastal pools (1). *E. moshkovskii* is osmotolerant, can be cultured at room temperature, and is resistant to emetine, all characteristics that distinguish it from *E. histolytica* and *E. dispar* (2–5). Human isolates of *E. moshkovskii* to date have come from North America, Italy, South Africa, and Bangladesh, and they have never been associated with disease (5,6). However, few studies have actually set out to identify such infections (7).

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The structural resemblance of the apparently innocuous *E. moshkovskii* to the disease-causing *E. histolytica* makes differentiating the two species important. In the clinical setting, for example, an *E. moshkovskii*–infected patient could be diagnosed as infected with *E. histolytica* and be treated unnecessarily with antiamebic chemotherapy. Most studies that have investigated the prevalence of *E. histolytica* and *E. dispar* have not considered the possible presence of *E. moshkovskii,* partly because of a lack of tools to detect *E. moshkovskii* other than cultivation, which is labor-intensive, not always successful, and problematic in the case of mixed infections. We report for the first time the application of tools to detect the species directly in stool and investigate the prevalence of *E. moshkovskii* in humans, a group of children in an *E. histolytica*– and *E. dispar*–endemic area where the first human infection with *E. moshkovskii* from Bangladesh was detected (6).

Materials and Methods

Stool Specimens

Fecal specimens included in this study were from 109 preschool children ages 2–5 years from Mirpur, an urban slum in Dhaka, Bangladesh. Based on results of polymerase chain reaction (PCR) on stool DNA samples, 39 were *E. dispar*–positive, 17 were *E. histolytica*–positive, and 1 was positive for both *E. histolytica* and *E. dispar*. Of the 52 samples negative by stool PCR, 18 were eventually found positive for *E. histolytica*, *E. dispar,* or both, either by PCR from culture DNA or by antigen detection tests performed on stool specimens, and the remaining 34 samples were negative by all methods. Only four of the samples were from children with diarrhea.

Cell Culture and Isoenzyme Analysis

All stool samples were cultured for *Entamoeba* species in Robinson's medium (8) within 6 hours of collection, and hexokinase isoenzyme analysis was performed when possible as previously described (9). *E. moshkovskii* strains Laredo and FIC were maintained axenically in LYI-S-2

[1]These authors contributed equally to this work.

medium (10) with 10% adult bovine serum. Laredo (ATCC 30042) is a human isolate, and FIC (ATCC 30041) is an environmental isolate. *E. histolytica* HM-1:IMSS clone 9 (ATCC 50528) and *E. dispar* SAW760 (ATCC 50484) were used as controls.

Antigen Detection Tests for *E. histolytica* and *E. dispar*

The TECHLAB, Inc. (Blacksburg, VA) *Entamoeba* test (designed to detect but not differentiate *E. histolytica* and *E. dispar* antigen in stool specimens) and *E. histolytica* test (designed to detect specifically *E. histolytica* in stool specimens) were performed on stool specimens according to the manufacturer's instructions (9).

Preparation of DNA

Stool DNA was isolated by using a modified version of the silica-DNA binding method of Katzwinkel-Wladarsch et al. as previously described (11,12). Culture DNA was isolated by a cetyltrimethylammonium bromide (CTAB) extraction method as previously described (13), dissolved in 10 mM Tris-Cl (pH 8.5), and passed over a Microspin S-200 HR column (Amersham Biosciences UK Ltd, Chalfont St. Giles, England). RNA was removed by the addition of RNase A (Promega UK, Ltd, Southampton, England) to 0.05 μg mL^{-1}.

Small Subunit rRNA Gene Amplification

Based on the sequences of the small subunit rRNA genes (SSU-rDNA) of *E. histolytica* and *E. dispar*, nested sets of primers (designated E-1/E-2, Eh-1/Eh-2, and Ed-1/Ed-2) were used, as described (11), to detect *E. histolytica* and *E. dispar* in stool specimens (Table 1). Based on the sequence of the SSU-rDNA gene of *E. moshkovskii* Laredo (GenBank accession no. AF 149906), a nested set of primers (designated Em-1/Em-2 and nEm-1/nEm-2) was designed (unpub. data) and used to detect *E. moshkovskii* in stool DNA (Table 1). In the initial PCR (total vol. 25 μL), 1.0 μL of stool or culture DNA was used. Thermal cycler conditions included 30 cycles, each consisting of 92°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a final extension of 7 min at 72°C. In the nested PCR, 1.0 μL of first PCR product was used as the template DNA and the annealing temperature was raised to 62°C, leaving the other parameters of the amplification cycles unchanged. *E. moshkovskii*–specific nested SSU-rDNA gene amplification products were digested with restriction endonuclease *Xho*I for 1 h at 37°C according to the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA) to verify species identity. All PCR products were separated in 1.8% NuSieve 3:1 agarose gels (Flowgen, Lichfield, England) in 1x Tris-borate-EDTA buffer and visualized after staining with ethidium bromide (0.2 μg mL^{-1}; Sigma-Aldrich Co. Ltd, Poole, England).

Table 1. Oligonucleotide primers used to detect *Entamoeba histolytica, E. moshkovskii,* and *E. dispar* in stool specimens

Primer	Primer sequence (5′ to 3′)
E-1	TTT GTA TTA GTA CAA A
E-2	GTA [A/G]TA TTG ATA TAC T
Eh-1	AAT GGC CAA TTC ATT CAA TG
Eh-2	TTT AGA AAC AAT GCT TCT CT
Ed-1	AGT GGC CAA TTT ATG TAA GT
Ed-2	TTT AGA AAC AAT GTT TCT TC
Em-1	CTC TTC ACG GGG AGT GCG
Em-2	TCG TTA GTT TCA TTA CCT
nEm-1	GAA TAA GGA TGG TAT GAC
nEm-2	AAG TGG AGT TAA CCA CCT
Arg^{TCT}-1	AGC ATC AGC CTT CTA AGC TG
Arg^{TCT}-2	CTT CCG ACT GAG CTA ACA AG
EmR-1	GGC GCC TTT TTT ACT TTA TGG
EmR-2	GCT AAC AAG GCC AAT CGA TAA A

Arg^{TCT} Gene PCR Amplification

Based on the sequence of the Arg^{TCT} tRNA gene of *E. histolytica*, a set of primers were designed (Arg^{TCT}-1 and Arg^{TCT}-2). Thermal cycler conditions for PCR were the following: 30 cycles each, consisting of 94°C for 1 min, 55°C for 1 min 30 s, and 72°C for 2 min, followed by a final extension of 5 min at 72°C. The Arg^{TCT} amplification products from *E. moshkovskii* Laredo, *E. moshkovskii* MS15-3646 (one of the infections detected above), and *E. dispar* SAW760 were cloned into the pGEM-T Easy vector (Promega) and sequenced (MWG Biotech Ltd, Milton Keynes, England). From the sequence results, an *E. moshkovskii*–specific primer pair, EmR-1 and EmR-2, was designed to amplify the *E. moshkovskii* Arg^{TCT} gene fragment specifically (Table 1). PCR amplification was performed at an annealing temperature of 58°C as described for Arg^{TCT} gene amplification.

Results

Culture and Isoenzyme Analysis

All 109 stool specimens were added to Robinson's medium for growth of *Entamoeba* species. Incubation led to growth of *E. histolytica*/*E. dispar*/*E. moshkovskii* in 33 cultures and *E. coli* in 8 cultures (no growth of *E. hartmanni* or *Endolimax nana* was observed). Hexokinase isoenzyme analysis was possible for 10 cultures; 4 of them showed the band pattern of *Entamoeba histolytica*, 5 showed *E. dispar,* and 1 showed the band pattern of *E. dispar* with an extra band just behind the faster moving band, perhaps indicating a mixed culture with *E. moshkovskii*.

Detection of *E. moshkovskii* by Nested PCR

The reference strain *E. moshkovskii* Laredo gave the expected band at approximately 260 bp with the *E. moshkovskii*–specific SSU-rDNA nested primers, whereas

control *E. histolytica* HM-1:IMSS and *E. dispar* SAW760 DNAs were negative. Twenty-three of 109 (21%) stool DNA samples were positive by nested PCR for *E. moshkovskii* (Table 2). Of these, seven were positive for amoebae by culture; one DNA sample extracted from these cultures was positive for *E. moshkovskii*. Seventeen of the 23 *E. moshkovskii*–positive samples were also positive for *E. histolytica, E. dispar,* or both, by either PCR of stool SSU rDNA (13/17) or by TECHLAB *Entamoeba* or *E. histolytica* tests (15/17) (Figure 1). One of the four children with diarrhea was positive for *E. moshkovskii* and coinfected with *E. dispar.* The cause of his diarrhea remained undetermined.

A comparison of SSU-rDNA sequences from *E. moshkovskii*, *E. histolytica*, and *E. dispar*, showed that the restriction endonuclease *Xho*I cuts exclusively in the *E. moshkovskii*–specific, 258-bp–nested PCR product to produce 236-bp and 22-bp fragments. Products from all 23 positive stool samples and the Laredo strain showed the presence of this site (Figure 1).

Table 2. Nested SSU rDNA polymerase chain reaction (PCR) (for *Entamoeba histolytica, E. dispar*, or both) and stool antigen-detection test results of the 17 *E. moshkovskii*–positive samples[a]

Samples	Stool antigen-detection test results	SSU rRNA gene PCR for *E. histolytica/E. dispar*	
		Stool DNA	Culture DNA
1[b]	*E. histolytica*	*E. dispar*	Mixed
2	*E. dispar*	0	NC
3	*E. dispar*	*E. dispar*	NC
4	*E. histolytica*	0	NC
5	*E. dispar*	*E. dispar*	*E. dispar*
6[b]	*E. dispar*	Mixed	*E. dispar*
7	*E. dispar*	*E. dispar*	NC
8	*E. dispar*	*E. dispar*	NC
9	*E. dispar*	*E. dispar*	NC
10	0	*E. dispar*	NC
11	*E. dispar*	*E. dispar*	NC
12	*E. dispar*	0	NC
13[c]	*E. dispar*	*E. histolytica*	NC
14	0	0	*E. dispar*
15	*E. dispar*	0	*E. dispar*
16	0	0	*E. dispar*
17	*E. histolytica*	0	NC
18	0	0	NC
19	0	0	NC
20	0	0	NC
21	0	0	NC
22	0	0	NC
23	0	0	NC

[a]NC, no culture; 0, negative. All stool antigen tests that are positive for *E. histolytica* can also be mixed because no specific *E. dispar* antigen test exists.

[b]Patients 1 and 6 likely had mixed infections with *E. histolytica* and *E. dispar*, in which *E. histolytica* was much lower in number than *E. dispar* in the stool specimen. For patient 1, SSU rDNA PCR failed to detect *E. histolytica*, though both species grew in the culture. For patient 6, although SSU rDNA PCR could detect *E. histolytica* in stool DNA, the *E. histolytica* antigen-detection test failed to detect *E. histolytica,* and only *E. dispar* survived in the culture.

[c]The stool specimen of patient 13 was marginally negative by the *E. histolytica* antigen-detection test (optical density value was 0.13 where the cut-off value for a positive result was 0.15).

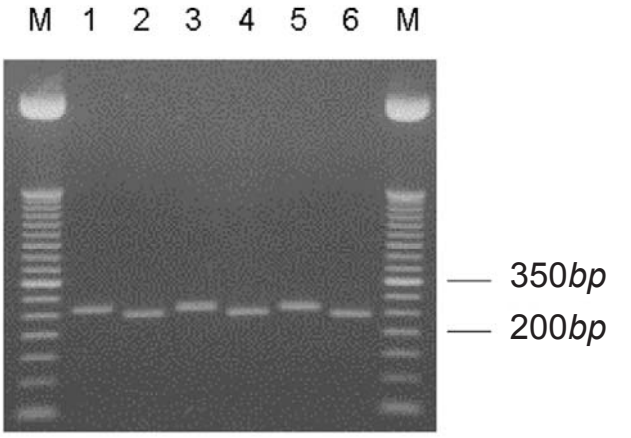

Figure 1. *Entamoeba moshkovskii*–specific nested SSU rDNA polymerase chain reaction (PCR) products. Odd- and even-numbered lanes represent undigested and XhoI-digested PCR products, respectively. Lanes 1/2, *E. moshkovskii* Laredo; lanes 3/4–5/6, DNA from stool samples. M, a 50-bp DNA ladder (Invitrogen Corp.).

Arg^{TCT} PCR and Sequence Analysis

To detect polymorphism among the *E. moshkovskii* samples, we studied a locus known to show polymorphism in *E. histolytica* and *E. dispar* (unpub. data). The Arg^{TCT} primers amplify *E. histolytica*, *E. dispar,* and *E. moshkovskii* DNA. The sizes of the PCR products from *E. histolytica* HM-1:IMSS, *E. dispar* SAW760, and *E. moshkovskii* Laredo were 586 bp, 586 bp, and 323 bp, respectively. We did not observe a band in the 250- to 350-bp region in any of the *E. histolytica* and *E. dispar* strains with these primers (data not shown). Because 17 of 23 *E. moshkovskii*–positive samples were also positive for *E. histolytica, E. dispar* (by SSU-rDNA PCR or TECHLAB enzyme-linked immunosorbent assay), or both, we ignored products in the 500- to 600-bp region (assuming that they were derived from *E. histolytica* or *E. dispar* DNA) and considered a sample positive for *E. moshkovskii* when it produced a band at approximately 300 bp. By this criterion, we found 18 of 23 samples were positive for *E. moshkovskii*, and they showed slight PCR product size variation (data not shown). The PCR products from one stool sample, *E. moshkovskii* Laredo and *E. dispar* SAW760, were cloned, sequenced, and aligned with that of *E. histolytica* HM-1:IMSS, and *E. moshkovskii*–specific primers (EmR-1 and EmR-2) were designed (Figure 2). In addition to notable PCR product size differences, analysis clearly showed that the *E. moshkovskii* sequence is completely different from those of *E. histolytica* and *E. dispar* and, unlike the *E. histolytica* and *E. dispar* sequences, it

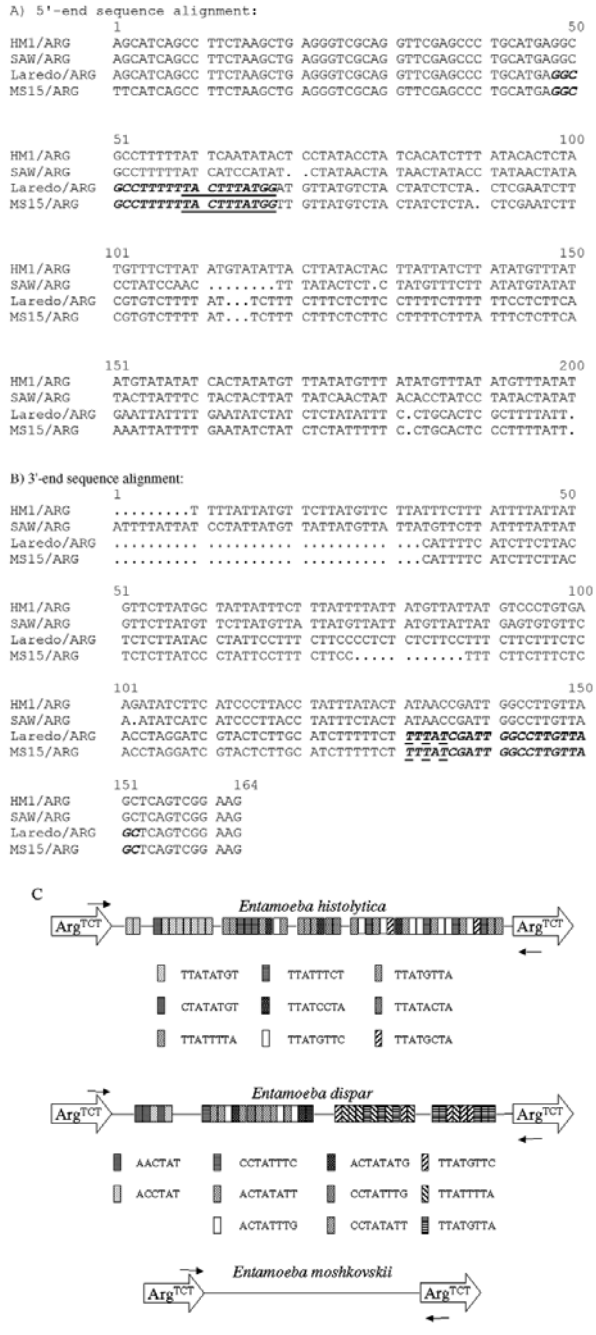

Figure 2. Arg^{TCT} locus. Arg^{TCT} sequences from *Entamoeba histolytica* HM-1:IMSS (GenBank accession no. AZ535059), *E. dispar* SAW760 (GenBank accession no. AF 525284), *E. moshkovskii* Laredo (GenBank accession no. AF 525285), and MS15-3646 (GenBank accession no. AF525286) were aligned at the 5′(A) and 3′ (B) ends to design *E. moshkovskii*–specific primers. The EmR primer sequences are shown in italic and bold with *E. moshkovskii*–specific positions underlined. C. Schematic representation of Arg^{TCT} loci from *E. histolytica* HM-1:IMSS, *E. dispar* SAW760, and *E. moshkovskii*. Locations of the primers used in polymerase chain reaction amplification are indicated by small arrows, the tRNA genes are indicated by large arrows, and the short tandem repeats by shaded boxes (not to scale.)

contains no short tandem repeat sequences (Figure 2C).

The EmR primers amplified the expected 265-bp fragment from *E. moshkovskii* Laredo DNA and did not amplify *E. histolytica* HM-1:IMSS or *E. dispar* SAW760 DNA. However, they successfully amplified 10 of a possible 23 *E. moshkovskii*–positive stool DNA samples. The most likely reason why these primers did not amplify the other 13 *E. moshkovskii* DNA samples is that they differed in sequence in the primer-binding regions. Although the PCR product size of the 10 positive samples was slightly different from that of Laredo, they were very similar in size to each other (Figure 3). The DNA of the previously reported *E. moshkovskii* ICDDRB:717, isolated from humans in the same geographic location (6), also gave a product of the same size (Figure 3, lane 2). The EmR primers successfully amplified DNA from environmental *E. moshkovskii* isolate FIC, but its product size was quite different from that of the human isolates of *E. moshkovskii* (Figure 3, lane 7).

Discussion

The main objectives of this study were to develop molecular tools to identify *E. moshkovskii* and to investigate its prevalence and diversity in humans. We were successful in developing a simple diagnostic technique: a nested SSU-rDNA PCR followed by restriction endonuclease digestion. We chose to use nested PCR to detect *E. moshkovskii* infections because our previous experience in this area showed that nested PCR was much more efficient in amplifying stool DNA (14). Our attempt to produce a species-specific polymorphic marker was not completely successful. The EmR primers failed to amplify 13 of 23 *E. moshkovskii*–containing samples, probably because of sequence differences in primer-binding sites. However, the Arg^{TCT} primers, originally designed to amplify *E. histolytica* and *E. dispar* DNA, did amplify most of the *E. moshkovskii* samples, producing a product distinct in size from those of *E. histolytica* and *E. dispar*.

Our study has some limitations. The subjects were children 2–5 years of age, so we do not know whether these subjects are representive of all age groups. All previous human isolates of *E. moshkovskii* have belonged to ribodeme 2 (5). Our attempts to perform riboprinting on these infections were unsuccessful, likely because of the size of the amplification target (approximately 1.95 kb). Even if PCR had been successful, the presence of mixed infections with other eukaryotes would have prevented successful typing.

This study has several important findings. The overall *E. moshkovskii* prevalence (21%) suggests that this infection is common among these children. *E. dispar*–infected children were almost twice as likely to have a mixed infection with *E. moshkovskii* (35%) compared to those with (18%) or without *E. histolytica* (18%) infections. None of

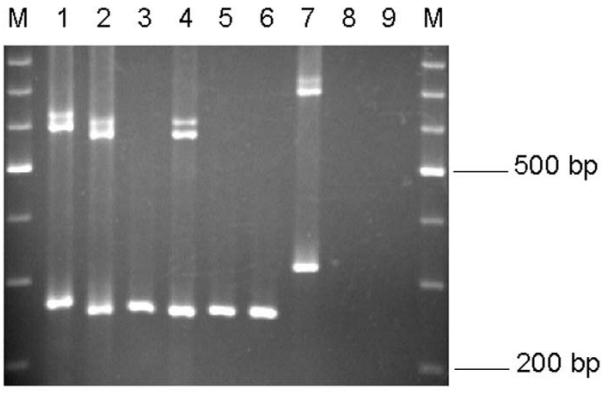

Figure 3. EmR polymerase chain reaction products. Lane 1, *Entamoeba moshkovskii* Laredo; lane 2, *E. moshkovskii* ICDDRB:717; lanes 3–6, *E. moshkovskii*–positive stool DNA samples; lane 7, *E. moshkovskii* FIC; lane 8, *E. histolytica* HM-1:IMSS; and lane 9, *E. dispar* SAW760. M, a 100-bp DNA ladder (Promega).

the six children with *E. moshkovskii* monoinfections had diarrhea or dysentery, which suggests that *E. moshkovskii* is a noninvasive parasite. The high prevalence of *E. moshkovskii* infection may have been unnoticed over the years because most such infections (74%) were mixed infections with *E. histolytica*, *E. dispar*, or both. Previous attempts to identify human *E. moshkovskii* infections (7) may have failed because the human intestinal flora was unsuitable for cultivation at room temperature.

The high prevalence of *E. moshkovskii* shown in this study population indicates that perhaps humans are a true host for this putatively free-living amoeba and are not just transiently infected. This prevalence may also explain some of the microscopy-positive/antigen-negative results obtained when using the *Entamoeba* test kit (15). Epidemiologic studies of *E. histolytica* infection should include tools to diagnose all three of these species individually, simultaneously, and accurately, and the prevalence of *E. moshkovskii* infection in other regions of the world should be investigated.

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Variant *Salmonella* Genomic Island 1 Antibiotic Resistance Gene Cluster in *Salmonella enterica* Serovar Albany

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Salmonella genomic island 1 (SGI1) contains an antibiotic resistance gene cluster and has been previously identified in multidrug-resistant *Salmonella enterica* serovars Typhimurium DT104, Agona, and Paratyphi B. We identified a variant SGI1 antibiotic-resistance gene cluster in a multidrug-resistant strain of *S. enterica* serovar Albany isolated from food fish from Thailand and imported to France. In this strain, the streptomycin resistance *aadA2* gene cassette in one of the SGI1 integrons was replaced by a *dfrA1* gene cassette, conferring resistance to trimethoprim and an open reading frame of unknown function. Thus, this serovar Albany strain represents the fourth *S. enterica* serovar in which SGI1 has been identified and the first SGI1 example where gene cassette replacement took place in one of its integron structures. The antibiotic resistance gene cluster of serovar Albany strain 7205.00 constitutes a new SGI1 variant; we propose a name of SGI1-F.

Multidrug-resistant *Salmonella enterica* serovar Typhimurium definitive phage type 104 (DT104) emerged during the 1980s as a global health problem because of the strain's involvement in diseases in animals and humans (1). Multidrug-resistant strains of this phage type were first identified from exotic birds in the United Kingdom in the early 1980s and in cattle and humans in the late 1980s; they have since become common in other animal species such as poultry, swine, and sheep. The DT104 epidemic has now spread worldwide, including several outbreaks since 1996 in the United States and Canada (2–5).

Multidrug-resistant *S. enterica* serovar Typhimurium DT104 is commonly resistant to ampicillin (Ap), chloramphenicol/florfenicol (Cm/Ff), streptomycin/spectinomycin (Sm/Sp), sulfonamides (Su), and tetracyclines (Tc). The antibiotic resistance genes are clustered in part of a 43-kb genomic island called *Salmonella* genomic island 1 (SGI1), located between the chromosomal *thdf* and *int2* genes (6,7). The *int2* gene is part of a retron that has been detected only in serovar Typhimurium (7). Downstream of the retron sequence is the *yidY* gene, which is also found in the chromosome of other *S. enterica* serovars (7). The antibiotic resistance gene cluster represents approximately one third of SGI1 and is located at the 3′ end of the structure (6,7). All resistance genes are clustered and are bracketed by two integron structures (Figure 1) (6–10). The first integron carries the *aadA2* gene, which confers resistance to Sm and Sp, and a truncated *sul1* (*sul1delta*) gene. The second integron contains the β-lactamase gene *pse-1* conferring resistance to Ap and a complete *sul1* gene conferring resistance to Su. Flanked by these two integron structures are the *floR* gene (8), also called *floSt* (11) or *cmlA*-like (9), which confers cross-resistance to Cm and Ff, and the tetracycline-resistance genes *tetR* and *tet*(G).

Recently, SGI1 has also been identified in other serovar Typhimurium phage types (i.e. DT120) and in other S. enterica serovars (i.e. Agona and Paratyphi B), indicating the horizontal transfer potential of SGI1 (6,10,12-15). In serovars Agona and Paratyphi B, SGI1 has the same chromosomal location as in serovar Typhimurium DT104, except that they lack the retron sequence found downstream of SGI1; thus it is located between the *thdf* gene and the *yidY* gene of their chromosomes (6,14). Moreover, variant SGI1 antibiotic resistance gene clusters have recently been reported for serovars Typhimurium DT104 and Agona (12,15). These clusters were probably generated after chromosomal recombinational events, resulting in either deletion or inactivation of some antibiotic resistance genes or in insertion of a new antibiotic resistance gene cassette. In particular, the *dfrA10* gene coding for trimethoprim (Tm) resistance was found downstream of the *pse-1* integron in two of the SGI1 variant antibiotic resistance gene clusters reported (15). They were accordingly classified in SGI1-A to -E; the resulting antibiotic resistance phenotypes were

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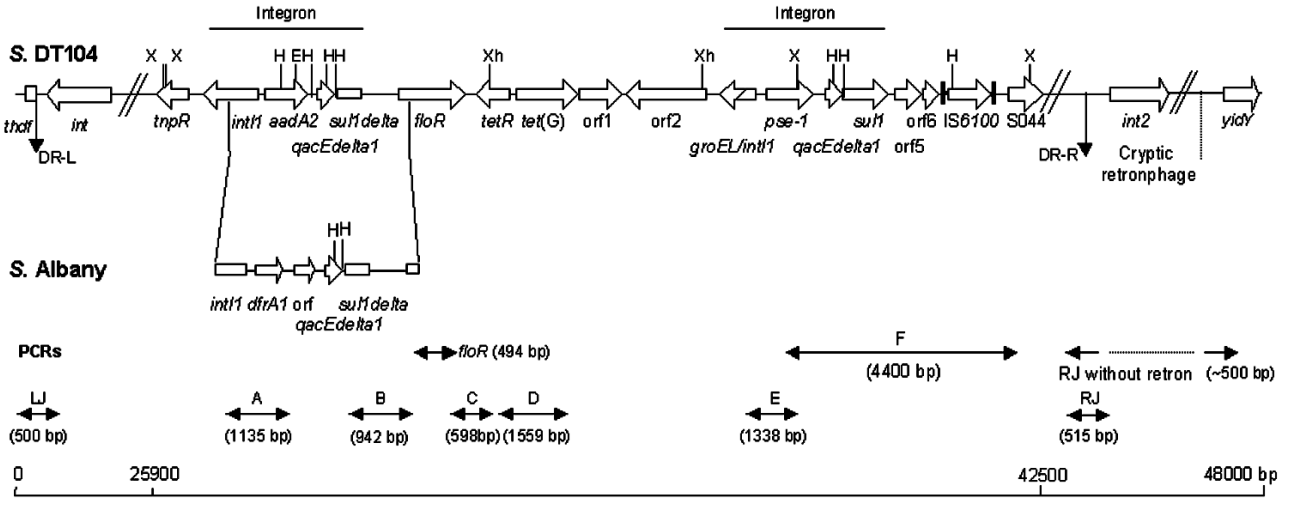

Figure 1. Genetic organization of the antibiotic resistance gene cluster of *Salmonella* genomic island 1 (SGI1) of *Salmonella enterica* serovar Typhimurium DT104 and serovar Albany strain 7205.00. DR-L and DR-R are the left and right direct repeats, respectively, bracketing SGI1. Polymerase chain reactions (PCRs) used to assess the genetic organization of the antibiotic resistance genes (PCRs *floR*, A, B, C, D, E, and F) and the SGI1 junctions to the chromosome (PCRs LJ and RJ for left and right junctions respectively) are indicated. Abbreviations used: *S.*, *Salmonella*; X, *Xba*I; H, *Hind*III; Xh, *Xho*I; E, *Eco*RI; orf, open reading frame.

ApCmFfSmSpSuTcTm, ApSu, SmSpSu, SmSpSuTm, and ApSmSpSuTc, respectively (15).

We examined a strain of *S. enterica* serovar Albany, isolated from food fish from Thailand and imported in France, that displayed the multidrug-resistance profile ApCmFfSuTcTm. This multidrug-resistance profile suggested the possible occurrence of SGI1 with a new variant antibiotic resistance cluster in this serovar.

Materials and Methods

The *S. enterica* serovar Albany strain 7205.00 used in this study was isolated from a food fish from Thailand. This strain and control strains *S.* Typhimurium DT 104 BN9181 (8,13,14), *S.* Agona 959SA97 (8,13,14), *S.* Paratyphi B 44 (14), and *Escherichia coli* strain TOP10 (Invitrogen SARL, Cergy-Pontoise, France) used in cloning experiments were grown at 37°C in brain heart infusion broth or agar plates. The strains were tested for their antibiotic susceptibility by the disc-diffusion assay on Mueller-Hinton plates. Susceptibility was tested by using discs containing the following antibiotics: Ap (10 µg), Cm (30 µg), Ff (30 µg), Sm (10 IU), Sp (100 µg), Su (200 µg), Tc (30 IU), and Tm (5 µg). All antibiotic disks except for Ff were purchased from Bio-Rad (Marnes-la-Coquette, France). Ff disks and the drug itself were obtained from Schering-Plough Animal Health (Kenilworth, NJ). MICs of Ff and Cm were determined by using the standard agar doubling dilution method. MIC breakpoints for Cm and Ff were defined by the Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM) or by the manufacturer (i.e., susceptible [MIC ≤8 µg/mL], intermediate [MIC = 16 µg/mL], or resistant [MIC ≥32 µg/mL]).

Detection of SGI1 and its location were performed by using primers corresponding to left and right (with or without retron) junctions in the chromosome as described (Table; Figure 1) (6,7,14). Polymerase chain reaction (PCR) mapping of the typical antibiotic resistance genes and integrons associated with SGI1 was performed by using conditions and primers as described (Table; Figure 1) (13–15). The antibiotic resistance gene organization was also assessed by Southern blots of genomic DNA cut by either *Xba*I, *Xho*I, *Hind*III or *Eco*RI by using as a probe the *Xba*I insert from recombinant plasmid pSTF3, comprising nearly the entire DT104 antibiotic resistance gene cluster, as described (13,14). Presence of SGI1 regions outside the antibiotic resistance gene cluster was assessed by Southern blot of genomic DNA cut by *Xba*I by using probe p1-9 as described (6,14). This probe contains a 2-kb *Eco*RI insert corresponding to a central region of SGI1, comprising parts of S023 and S024 open reading frames (ORFs), which code for putative helicase and exonuclease proteins (6).

PCR amplification of the first integron was also performed by using primer int1 of PCR A and primer F3 of PCR B (Figure 1). Cloning of this PCR product in plasmid pCR2.1-TOPO was performed by using the TOPO TA cloning kit (Invitrogen). We used Genome Express (Meylan, France) for nucleotide sequencing of the insert.

Genomic DNA, contained in agarose plugs, was digested with *Bln*I or *Xba*I and fragments separated by pulsed-field gel electrophoresis (PFGE) performed by using the CHEF-DR III system (Bio-Rad, Hemel Hempstead, U.K.) at 6 V/cm for 24 h with pulse times of 10–30 sec. The nucleotide sequence of the *S.* Albany strain 7205.00 integron fragment harboring the Tm resistance

Table. Primers used for polymerase chain reaction

Primer	Gene	Amplification[a]	Size (bp)	Nucleotide sequence (5′–3′)
U7-L12	*thdf*	Left junction	500	ACACCTTGAGCAGGGCAAG
LJ-R1	*int*			AGTTCTAAAGGTTCGTAGTCG
104-RJ	S044	Right junction		TGACGAGCTGAAGCGAATTG
C9-L2	*int2*		515	AGCAAGTGTGCGTAATTTGG
104-D	*yidY*		500	ACCAGGGCAAAACTACACAG
cml01	*floR*	*floR*	494	TTTGGWCCGCTMTCRGAC
cml15	*floR*			SGAGAARAAGACGAAGAAG
int1	*intI1*	A	1,135	GCTCTCGGGTAACATCAAGG
aad	*aadA2*			GACCTACCAAGGCAACGCTA
sulTER	*sul1delta*	B	942	AAGGATTTCCTGACCCTG
F3	*floR*			AAAGGAGCCATCAGCAGCAG
F4	*floR*	C	598	TTCCTCACCTTCATCCTACC
F6	*tetR*			TTGGAACAGACGGCATGG
tetR	*tetR*	D	1,559	GCCGTCCCGATAAGAGAGCA
tetA	*tetA*			GAAGTTGCGAATGGTCTGCG
int2	*groEL-intI1*	E	1,338	TTCTGGTCTTCGTTGATGCC
pse1	*pse-1*			CATCATTTCGCTCTGCCATT
pse-L	*pse-1*	F	4,400	AATGGCAATCAGCGCTTCCC
MDR-B	S044			GAATCCGACAGCCAACGTTCC

[a]See Figure 1.

gene has been deposited in GenBank under accession number AY146989.

Results

Multidrug-Resistant Phenotype

S. Albany strain 7205.00 showed a multidrug-resistant phenotype similar to SGI1 carrying *S. enterica* serovars Typhimurium DT104, Agona, or Paratyphi B, (i.e., Ap, Cm/Ff, Su, Tc). The strain was susceptible to Sp and Sm but showed additional resistance to Tm. The strain showed the same level of resistance to Ff as *S. enterica* serovars Typhimurium DT 104, Agona, or Paratyphi B with a Ff MIC of 64 µg/mL. No plasmids were detected in the *S.* Albany strain, suggesting a chromosomal location of antibiotic-resistance genes and possibly the presence of SGI1.

Identification of SGI1

To assess the presence of SGI1 and its location in the chromosome of the *S.* Albany strain 7205.00, PCR was performed by using primers corresponding to the left and right junctions of SGI1 in the *Salmonella* chromosome (Figure 1). We also used PCR to detect the presence or absence of the *int2*-retron sequence, which is located downstream of SGI1 in serovar Typhimurium DT104 but not in serovars Agona and Paratyphi B (6,14). PCR results were positive for the left junction of SGI1, as for the other serovars. If a sequence of the *int2* gene of the retron was used as reverse primer, the PCR results were negative for the right junction of SGI1 but positive if the sequence of the *yidY* gene was used. PCR products showed the expected sizes of approximately 500 bp for both the left junction and right junction PCR without the retron sequence, as indicated in Figure 1. These data thus indicate that the serovar Albany strain 7205.00 contains SGI1 at the same chromosomal location as in serovars Typhimurium DT104, Agona, or Paratyphi B (i.e., between the *thdf* and *yidY* genes) but lacks the retron sequence found in DT104 and other serovar Typhimurium strains (6,7).

The nucleotide sequences of the left and right junction PCR products were determined, allowing an analysis of the imperfect 18-bp direct repeats flanking SGI1 (6,7). This direct repeat appeared to be a duplication of the last 18 bp of the *thdf* gene. The right junction direct repeat sequence (DR-R) was previously shown to be identical to the sequence from the respective *thdf* sequences from sensitive serovar Typhimurium or Agona strains, suggesting the origin of the DR-R is actually the end of *thdF* (Figure 2) (6). These sequences are slightly divergent between serovars Typhimurium and Agona, with a C

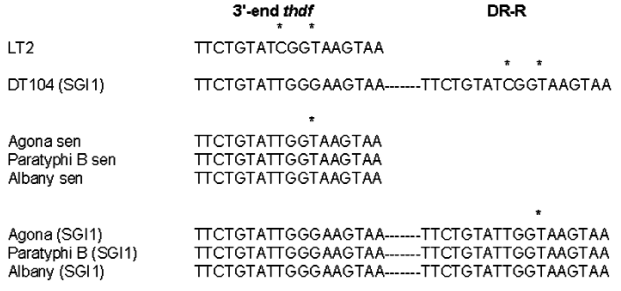

Figure 2. Alignment of the direct repeats (DR) flanking the *Salmonella* genomic island 1 (SGI1) in serovars Typhimurium DT104 (DT104), Agona, Paratyphi B, and Albany. Asterisks represent nucleotide substitutions. Sen, sensitive.

located at position 9 of the direct repeat in serovar Typhimurium as opposed to a T at this position in Agona. The left junction direct repeat sequence (DR-L) is identical in both serovars, suggesting the origin of this sequence may be from the donor DNA and not the result of a duplication event. As shown in Figure 2, DR-L and DR-R sequences in the serovar Paratyphi B strain, which were not investigated in a previous study (14), and in the serovar Albany strain 7205.00 are identical to those found in serovar Agona strains carrying SGI1 (Figure 2). These results reinforce the hypothesis that the SGI1 insertions in serovar Typhimurium DT104 and in the other serovars were separate events and not a result of genetic exchange between serovar Typhimurium DT104 and the other serovars.

Aside from the antibiotic resistance genes presented below, the presence of the entire SGI1 in the serovar Albany strain 7205.00 was confirmed by Southern blot of *Xba*I-digested genomic DNA with the p1-9 probe, as described previously. This probe showed *Xba*I fragments of the expected 4- and 9-kb sizes as in control serovar Typhimurium DT 104, Agona, and Paratyphi B strains carrying SGI1 (Figure 3A).

New Variant Antibiotic Resistance Gene Cluster

PCR mapping of the typical antibiotic resistance genes and integrons associated with SGI1 is schematized in Figure 1. PCR amplifications on genomic DNA extracted from serovar Albany strain 7205.00 yielded fragments B, C, D, E, F, and partial *floR* of the sizes expected from DNA of serovar Typhimurium DT104 control strain BN9181 (data not shown). However, fragment A specific for the *aadA2* integron was not obtained. Thus, these PCR mapping results indicated the presence of *floR*, *tetR*, and *tet*(G) genes and the second integron carrying the *pse-1* gene. A positive PCR result for fragment B, representing the link between the *aadA2* integron and *floR*, would nevertheless suggest the presence of at least a partial *aadA2* integron. These data are in accordance with the antibiotic resistance phenotype of serovar Albany strain 7205.00 (i.e., ApCmFfSuTc with lack of resistance to Sm and Sp) and indicate the presence of a SGI1 variant antibiotic resistance gene cluster at the level of the *aadA2* integron.

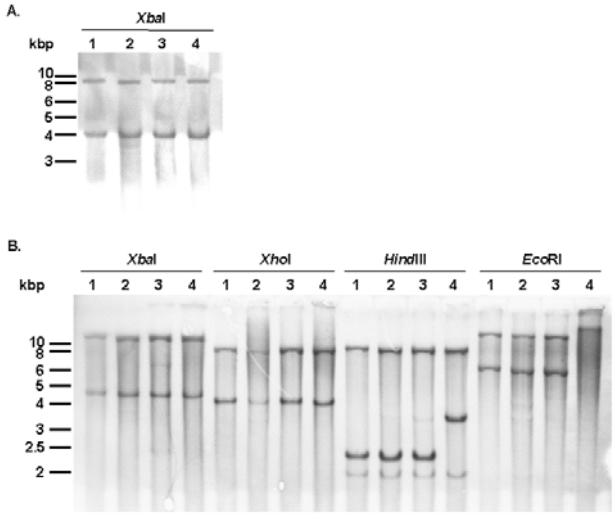

Figure 3. A) Southern blot hybridization with the p1-9 probe of *Xba*I-digested genomic DNAs of *Salmonella enterica* serovar Typhimurium DT104 strain BN9181 (lane 1), serovar Agona strain 959SA97 (lane 2), serovar Paratyphi B strain 44 (lane 3), and serovar Albany strain 7205.00 (lane 4). B) Southern blot hybridization of *Xba*I-, *Xho*I-, *Hind*III-, and *Eco*RI-digested genomic DNAs of serovar Typhimurium DT104 strain BN9181 (lanes 1), serovar Agona strain 959SA97 (lanes 2), serovar Paratyphi B strain 44 (lanes 3), and serovar Albany strain 7205.00 (lanes 4), with the pSTF3 probe containing all antibiotic resistance genes (see Figure 1).

These results were confirmed by Southern blot of genomic DNA digested by *Xba*I, *Xho*I, *Hind*III, or *Eco*RI with the pSTF3 probe containing nearly the entire antibiotic resistance gene cluster of serovar Typhimurium DT104 strain BN9181 (see *Xba*I fragment in Figure 1). *Xba*I and *Xho*I Southern blot profiles of the serovar Albany strain were similar to those obtained for the control strains of serovar Typhimurium DT104, Agona, and Paratyphi B harboring SGI1 (Figure 3B). However, the *Hin*dIII and *Eco*RI Southern blot profiles of the serovar Albany strain were clearly distinct from those of the control strains, confirming the presence of a variant antibiotic resistance gene cluster in the serovar Albany strain.

The genetic variation at the level of the *aadA2* integron in the serovar Albany strain was further assessed by PCR with a forward primer of fragment A and reverse primer of fragment B (Figure 1). This PCR result was positive and yielded a fragment approximately 300 bp larger than with DNA from serovar Typhimurium DT104 control strain BN9181 (data not shown). This PCR product was cloned in plasmid pCR2.1-TOPO and sequenced. *E. coli* carrying this plasmid were resistant to Tm, indicating the presence of a Tm resistance gene in the fragment. Sequence analysis (done by using BLAST [available from: URL: http://www.ncbi.nlm.nih.gov:80/BLAST/]) showed, in addition to the corresponding nucleotide sequence of the DT104 antibiotic resistance gene cluster, two gene cassettes (*dfrA1* coding for Tm resistance and an ORF of unknown function), described in class 1 integrons of *Vibrio cholerae* strains isolated in Thailand and India (99% nucleotide identity; GenBank accession nos. AF221901 and AF455254) (16,17). Thus, instead of the *aadA2* gene classically found in the first integron of the SGI1 antibiotic resistance gene cluster, *dfrA1* and an ORF of unknown function were found in the corresponding integron of serovar Albany strain 7205.00. The conserved regions of

this integron were 100% identical to those found in serovar Typhimurium DT104 with a truncated *sul1* gene (Figure 1). The distinct serovar Albany strain 7205.00 *Hin*dIII and *Eco*RI Southern blot profiles with probe pSTF3 described above are in accordance with the nucleotide sequence of the variable region containing *dfrA1* of this integron (Figure 1). The antibiotic resistance gene cluster of serovar Albany strain 7205.00 constitutes a new SGI1 variant; we propose a name of SGI1-F, according to previously proposed nomenclature (15).

Evidence for Horizontal Transfer

Macrorestriction analysis by PFGE of the serovar Albany strain DNA cut by *Xba*I or *Bln*I showed that the strain is genetically distinct from serovars Typhimurium DT104, Agona, and Paratyphi B in which SGI1 has been identified (Figure 4). This distinction further indicates at the molecular level that the occurrence of SGI1 in the serovar Albany strain probably results from horizontal transfer and not seroconversion of known *S. enterica* serovars harboring SGI1.

Discussion

SGI1 is the first genomic island containing an antibiotic resistance gene cluster identified in *S. enterica*; its acquisition in *S.* Typhimurium phage type DT104 was possibly an important trait in the worldwide epidemic of the resulting multidrug-resistant clone causing disease in animals as well as in humans. SGI1 has been further identified in other *S. enterica* serovars, such as in serovar Agona strains isolated from poultry in Belgium and in a serovar Paratyphi B strain isolated from a tropical fish in Singapore (6,13,14). The serovar Albany fish isolate from Thailand in this study represents the fourth *S. enterica* serovar in which SGI1 has been identified. The identification of SGI1 in several *S. enterica* serovars, shown by PFGE to be genetically distinct, suggests horizontal transfer of this region. That SGI1 has the same chromosomal location in the different serovars suggests that its insertion occured through site-specific recombination. Genes such as the tandemly arranged *int* and *xis* genes found adjacent to the DR-L of SGI1 may play a role in this recombination event because they encode a putative integrase and excisionase, respectively (6). Sequence analysis of the left and right junctions of SGI1 to the *Salmonella* chromosome provides additional clues about these recombination events in the different serovars. The right junction 18-bp DR-R direct repeat sequence found at the 3′ end of SGI1 appears to be a duplication of the last 18 bp of the *thdf* gene found upstream of SGI1. The left junction SGI1 18-bp DR-L direct repeat sequence is slightly different from the 3′-end of *thdf* in sensitive *S. enterica* serovars lacking SGI1

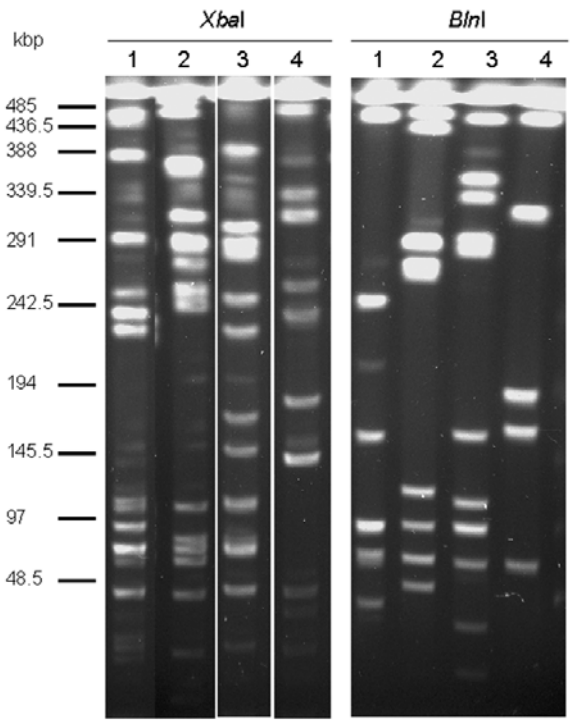

Figure 4. Macrorestriction analysis by pulsed-field gel electrophoresis of genomic DNAs cut by *Xba*I or *Bln*I of *Salmonella enterica* serovar Typhimurium DT104 strain BN9181 (lanes 1), serovar Agona strain 959SA97 (lanes 2), serovar Paratyphi B strain 44 (lanes 3), and serovar Albany strain 7205.00 (lanes 4).

(Figure 2) but identical in all serovars carrying SGI1. This finding suggests that the origin of this sequence may be from the donor DNA. When SGI1 insertion takes place in a sensitive *S. enterica* serovar, the last 18 bp of its *thdf* gene would be duplicated and found at the 3′ end of SGI1 and replaced by the 18 bp of the donor DNA at the 5′ end of SGI1. In other words, the last 18 bp of *thdf* in sensitive *S. enterica* serovars may constitute a hotspot for homologous recombination with an 18-bp similar sequence of the SGI1 donor DNA. These DR-R and DR-L minor sequence differences also support the hypothesis that the SGI1 insertions in serovar Typhimurium DT104 and other serovars were separate events and not a result of genetic exchange between serovar Typhimurium DT104 and the other serovars (6). Yet, the origin of SGI1 remains to be determined.

A similar situation has been reported for an approximately 100-kb genomic island in *V. cholerae* called SXT conjugative, self-transmissible, integrating (constin) element. It carries multiple antibiotic resistance genes, including *floR* as in SGI1 (18,19). Integration of the element has been experimentally shown to occur through site-specific recombination in a 17-bp sequence found in the circular form of the SXT element and a similar 17-bp

sequence of *prfC* of the *V. cholerae* and *E. coli* chromosomes (20). Chromosomal integration and excision of the SXT element required an element-encoded *int* gene that is found as first gene of the SXT element, as is the *int* gene of SGI1 (18,20). SGI1 may also exist in an intermediate circular form, but this remains to be demonstrated.

Most of the time, the antibiotic resistance gene cluster of SGI1 contains five antibiotic resistance genes (6–15). Variant SGI1 antibiotic resistance gene clusters have been recently reported in serovars Typhimurium DT104 and Agona containing part of the antibiotic resistance genes or an additional resistance gene (i.e., *dfrA10* coding for Tm resistance) (12,15). These variant antibiotic resistance gene clusters were probably generated by recombinational events such as deletions and insertions. The serovar Albany strain of our study represents the first SGI1 example in which gene replacement took place in one of the integron structures. The *dfrA1* and ORF gene cassettes found instead of *aadA2* may have been introduced by homologous recombination with a class 1 integron containing the same array of gene cassettes from another bacterium (21). Another possibility is the exchange between *aadA2* and the two gene cassettes, which would imply excision, mediated by the integron-encoded integrase, of *aadA2* and its replacement by the other gene cassettes (22). The array of gene cassettes found in the integron of the serovar Albany strain were the same as those recently reported in integrons of *V. cholerae* isolated in Thailand and India (16,17). Considering the origin of the serovar Albany strain (i.e., fish exported from Thailand), a possible explanation could be the exchange of antibiotic resistance gene cassettes between epidemic multidrug-resistant *V. cholerae* strains and *Salmonella* strains from Thailand. Moreover, during the choleralike epidemic among the Khmers in 1982, children and pregnant women were reportedly treated with trimethoprim-sulfamethoxazole (16,23). A high percentage of Khmer outbreak *V. cholerae* strains showed resistance to trimethoprim-sulfamethoxazole; the strains that acquired the *dfrA1* gene cassette likely became predominant through selective pressure (16,24).

The multidrug-resistant *V. cholerae* epidemics in humans in Asia might be largely responsible for spread of antibiotic resistance genes. Recent reports describe that human colonization by *V. cholerae* creates a hyperinfectious bacterial state, which is perpetuated even after purging into natural aquatic reservoirs and may contribute to epidemic spread of cholera (25). These aquatic reservoirs may be an ecologic niche where antibiotic resistance gene exchange takes place between different enterobacterial pathogens. In various seawater places around Hong Kong where untreated sewage is discharged, several enterobacterial pathogens were simultaneously detected such as *Salmonella* and *V. cholerae* (26).

As shown in the present study, gene replacement in the integron structures is another way to contribute to variability of the antibiotic resistance gene cluster of SGI1. SGI1 may thus serve as a vehicle of various antibiotic resistance genes in different *S. enterica* serovars, a situation somewhat similar to that reported for SXT constins and integrons of multidrug-resistant *V. cholerae* strains (16–20).

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Mr. Doublet is a doctoral student at the Institut National de la Recherche Agronomique in France. His main interests are antibiotic resistance mechanisms and the spread of antibiotic-resistance genes in enterobacterial pathogens.

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Emerging Rickettsioses of the Thai-Myanmar Border[1]

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To investigate the presence of rickettsioses in rural residents of the central Thai-Myanmar border, we tested the blood of 46 patients with fever. Four patients had murine typhus, three patients had scrub typhus, and eight patients had spotted fever group rickettsioses, including the first case of *Rickettsia felis* infection reported in Asia.

Human rickettsioses known to occur in Thailand include mainly murine typhus and scrub typhus. Murine typhus is caused by *Rickettsia typhi* and is primarily maintained by fleas such as *Xenopsylla cheopis,* with various rodents reservoirs (1). Scrub typhus is caused by *Orientia tsutsugamushi* (formerly named *R. tsutsugamushi*), which is transmitted by the bites of the larvae of several species of trombiculid mites (commonly called "chiggers") (2).

Spotted fever group (SFG) rickettsioses are associated with arthropods, mainly ticks but mites and fleas as well (3,4). In Thailand, few reports of serologically documented cases of SFG rickettsioses have been published (5). Although the specific etiologic agents of these diseases have not been identified, several SFG rickettsiae have been identified from ticks in Thailand. Thai tick typhus *Rickettsia* TT-118 was isolated from a pool of ticks in the 1970s (6). Despite its name, its pathogenic role in Thailand is not known. However, Stenos et al. have suggested that TT-118 is a strain of *R. honei,* an emerging pathogen prevalent on Flinders Island, Australia (7). Further, a rickettsia identified as *R. honei* type strain has also been recently detected by molecular methods in Thai *Ixodes granulatus* (8). In addition, several previously unrecognized rickettsiae of unknown pathogenicity have been detected from *Ixodes* and *Dermacentor* ticks, including species known to bite humans (8,9).

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Thailand's Sangkhlaburi District (Kanchanaburi Province) is a major gateway on the central part of the Thai-Myanmar border where newly arrived migrants from Myanmar become established as farm or factory laborers. There, the local Thai people, as well as Karen, Mon, and Burmese migrants, are commonly bitten by arthropods when working in the fields or at home. Scrub typhus has been previously reported in the province (10). Murine typhus was also described as a cause of fever in refugee camps along the Thai-Myanmar border (11). However, a serosurvey undertaken in 1997 suggested that residents of Sangkhlaburi were commonly exposed not only to the agents of scrub typhus and murine typhus but also to SFG rickettsiae and agents of human ehrlichioses (12). Here, we provide for the first time a more precise indication of rickettsioses in febrile patients from Sangkhlaburi.

The Study

This study was based at the Armed Forces Research Institute of Medical Sciences (AFRIMS)–Kwai River Christian Hospital Clinical Center, Sangkhlaburi District, Kanchanaburi Province, Thailand. (The protocol was approved by the Human Subjects Research Review Board of the U.S. Army, Ethical Review Committee for Research in Human Subjects of the Thai Ministry of Public Health, and Scientific Review Committee of AFRIMS.) Patients were selected from those enrolled and sampled from June 1999 to February 2002 in an on-going "fever study," which focuses on the etiology of undifferentiated febrile illnesses (oral temperature >38°C or history of fever within the past 48 h) in local residents ≥20 years of age. Criteria leading to the suspicion of rickettsioses included 1) a rash or eschar, 2) arthropod bites or recent exposure to the jungle, 3) a negative Giemsa-stained malaria smear, and 4) serum specimens that tested positive by enzyme-linked immunosorbent assay (ELISA) for SFG–specific immunoglobulin (Ig) M (PanBio, Brisbane, Australia) or dot-ELISA for total Ig of *R. rickettsii* or *R. typhi* (PanBio-INDX, Baltimore, MD). Serum specimens were sent to the Unité des Rickettsies in Marseille for specific diagnosis of rickettsioses. Serologic testing was performed by indirect immunofluorescence (IF) on acute-phase (day 0) and convalescent-phase (approximately day 21) samples. Serum specimens were tested by using a panel of 13 rickettsial antigens, including SFG rickettsiae (*R. conorii* Indian, *R. japonica*, *R. honei*, *R. helvetica*, *R. slovaca*, AT1 *Rickettsia* [13], *R. felis*, "*R. heilongjiangii"*) typhus group rickettsiae (*R. typhi*), *Orientia tsutsugamushi* (strain Gilliam, Kato, Karp, and Kawazaki), *Anaplasma phagocytophilum,*

[1]These results were presented in part at the 3rd International Conference on Emerging Infectious Diseases, March 24–27, 2002, Atlanta, Georgia, USA.

Ehrlichia chaffeensis, and *Coxiella burnetii*. The rationale for the antigen screening panel included the presence of the strains in Asia and results of previous serosurveys for *A. phagocytophilum* and *E. chaffeensis*. The standard procedure was followed for the use of Western blot and cross-adsorption studies to complete the IF assay at the Unité des Rickettsies (14,15). An immunofluorescence assay was considered positive for 1) IgG with titers ≥128 and/or IgM titers ≥64 for *R. conorii;* and 2) for IgG titers ≥64 and/or IgM titers ≥32 for other rickettsial antigens. When cross-reactions were noted between several rickettsial antigens, the standard procedure comprised three steps: 1) A rickettsial antigen was considered to represent the agent of infection when titers of IgG and/or IgM antibody against this antigen were at least two serial dilution higher than titers of IgG and/or IgM antibody against other rickettsial antigens. 2) When the difference in titers between several antigens was lower than two dilutions, Western blot assays were performed. A rickettsial antigen was considered to represent the agent of the infection when acute-phase or convalescent-phase sera reacted only with the specific proteins of this antigen. 3) When Western blot assays were not diagnostic, cross-absorption studies were performed: IgG/IgM titers had to be ≥128/32. Specific diagnosis criteria after cross-absorption studies included a) IF serologic test results positive for a single antigen or b) a Western blot assay showing an exclusive reactivity with specific proteins of a sole agent.

From June 1999 to February 2002, 46 patients were selected to be specifically tested for rickettsioses. These 46 patients were empirically treated by a 7-day doxycycline regimen (200 mg/d). Rickettsioses were serologically confirmed in 15 (33%) patients by evidence of seroconversion, IgM at significant titers, or both. Three patients (nos. 1–3) had scrub typhus caused by *O. tsutsugamushi*. Serum specimens from patients 1 and 3 provided the highest titers against Gilliam and Karp strains, and serum from patient 2 had titers against Gilliam strain only. No further study was conducted to identify the strain responsible for the disease. Two of these patients had returned from a trip into the jungle, and the third became sick several days after cutting grass in the fields. One patient was initially thought to have bacterial meningitis and had been treated unsuccessfully by a broad-spectrum third-generation cephalosporin for 3 days before doxycycline was started. Four patients (nos. 4–7) had murine typhus caused by *R. typhi*. All had fever and unspecific signs. The patients recalled no arthropod bite, and none had a rash. Eight cases were SFG rickettsioses (nos. 8–15). Of the patients with SFG rickettsioses, only one (no. 9) had fever, eschar, and rash. One patient (no.13) had an eschar at a tick bite site, and another had a rash (no.15). Others presented with unspecific signs. Cross-reactions were noted mostly within the SFG rickettsia antigens. One patient (no. 8) with SFG rickettsioses seroconverted to *R. felis*, indicated by high level of antibody titers. Further, although IgG titers were more than two serial dilutions higher than those for *R. typhi*, Western blot assay was performed to confirm IF findings. Two patients (nos. 9 and 10) were shown to have the highest titers to *R. conorii* strain Indian. Five patients (nos. 11–15) had the highest titers to *R. helvetica*. For patients 4, 9, 10, 13, and 15, IF results showed differences lower than two dilutions in IgG titers, IgM titers, or both, between several antigens. Thus, IF assays were completed by Western blot and with cross-absorption studies for patient 4 (Table). No cases of infection due to *C. burnetii* or ehrlichioses were diagnosed in the 46 tested patients.

Conclusions

In this study, we report rickettsioses in Sangkhlaburi, including the first case of *R. felis* infection reported in Asia. *R. felis* is an emerging pathogen responsible for flea-borne spotted fever. *R. felis* was likely first detected (as *R. ctenocephali*) in European cat fleas (*Ctenocephalides felis)* in 1918 (16), then rediscovered in 1990 in the United States (17). *R. felis* was then cultivated and characterized as a unique SFG rickettsia (18). Its pathogenic role was recently demonstrated in patients with serologic evidence of infection in Brazil, France, and Germany. *R. felis* DNA has also been detected in sera in Texas, Mexico, Brazil, and Germany (19). This rickettsia has also been recently detected in fleas in Brazil, Africa, Spain, and France (20). Further, during an entomologic survey, *R. felis*–like rickettsiae were detected in fleas collected in Sanghklaburi (P. Parola, unpub. data). These data suggest that *R. felis* infection is endemic in Sanghklaburi and perhaps globally.

Murine typhus, a mild disease with nonspecific signs (21), was found in four of our patients. Although this disease has a worldwide distribution, it is often unrecognised, and documented cases are rarely reported. The classic triad of fever, headache, and skin rash is observed in <15% of cases (22). For example, our four patients did not have a rash. Arthralgia, myalgia, and respiratory and gastrointestinal symptoms (as demonstrated by one of our patients) are frequent (21,22). Regarding disease transmission, although rats and mice are very common within and around houses in the villages, our patients did not report contact with rat fleas or a flea bite.

In this study, seven patients with SFG rickettsioses may have been infected by *R. helvetica* (five patients) or *R. conorii* Indian strain (two patients), according to IF assays completed for some cases by Western blot and cross-adsorption studies. *R. helvetica* is an emerging pathogen known to be prevalent in Europe (23) and Japan (13). In both areas, *R. helvetica* is associated with *Ixodes* ticks, which are also found in Thailand, although they have not

Table. Clinical and laboratory data of patients with rickettsioses on the Thai-Myanmar border

Patient no.	Age/ sex	A. bite	Clinical signs accompanying fever				Immunofluorescence serologic testing[a] IgG/IgM early IgG/IgM late												
							SFG rickettsia antigens								*R. typhi* antigen	*Orientia tsutsugamushi*			
			R	E	N	Other	Rh	Rc	Rjap	Rhon	Rslo	AT1	Rheil	Rfel		G	Kw	Kp	K
Scrub typhus																			
1	29/M	Not noticed	No	No	Yes	Headache, stupor, meningism, thrombocytopenia, ↑ALT	0/16 0/16	0/0 0/0	0/16 0/16	0/8 0/8	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/32 256/32	0/8 0/8	0/8 256/32	0/0 0/0
2	50/F	Yes	Yes[b]	Yes	No	Chills, myalgia, vomiting, thrombocytopenia, ↑GGT ↑ALT	0/32 0/32	0/0 0/0	0/32 0/32	0/16 0/16	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	128/64 128/16	0/0 0/0	0/0 0/0	0/0 0/0
3	32/M	Yes	No	No	Yes	Headache, myalgia, cough, thrombocytopenia, ↑GGT ↑ALT	0/16 16/16	0/0 0/0	0/0 16/16	0/8 0/8	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 512/128	0/0 0/0	0/0 512/0	0/0 0/0
Murine typhus																			
4	28/F		No	No	No	Chills, headache, vomiting, myalgia, cough, thrombocytopenia	16/128[e] 16/64[c]	16/64 16/64	16/8 0/0	16/16 16/64	16/16 16/8	0/0 0/0	0/32 0/8	0/0 0/0	**64/128**[e] **32/32**[e]	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
5	35/M	Not noticed	No	No	No	Chills, headache, ↑ALT	0/64 16/128	0/64 16/256	0/8 0/8	0/64 0/64	0/0 0/0	0/0 0/0	0/32 0/128	0/0 0/0	**16/256** **512/256**	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
6	37/F	Not noticed	No	No	No	Chills, headache, myalgia, vomiting, thrombocytopenia, ↑GGT ↑ALT	16/64 128/64	32/8 32/0	0/0 32/0	16/0 128/0	16/0 128/8	0/0 32/8	0/0 32/0	0/0 0/0	**64/8** **512/256**	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
7	20/F	Not noticed	No	No	No	Headache, back pain	0/128 16/64	0/64 0/64	0/0 0/64	0/32 0/64	0/0 0/164	0/0 0/8	0/0 0/0	0/0 0/0	**0/64** **128/256**	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
SFG rickettsioses																			
8	70/F		No	No	No	Chills, headache, vomiting, hepatomegaly, leukopenia	0/32 32/32	0/32 32/32	0/0 0/0	0/8 16/8	0/0 0/0	0/0 0/0	0/0 0/0	**0/0** **1024/256**[d]	0/0 0/256[d]	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
9	50/M	Not noticed	Yes[c]	Yes	Yes	Chills, abdominal pain, confusion, thrombocytopenia	64/8[d] 64/16	**128/16**[d] **128/16**	32/0[d] 32/0	32/0[d] 64/0	32/0[d] 128/0	0/0 0/0	16/0[d] 32/8	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
10	45/M		No	No	Yes	Vomiting, diarrhea, hepato-splenomegaly	16/32[d] 64/32	**64/32**[d] **64/32**	16/32[d] 32/16	16/0[d] 32/16	16/16[d] 16/16	0/0 0/0	16/8[d] 32/16	0/0 0/0	0/16[d] 16/32	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
11	35/M	Tick bite	No	Yes	No	Chills, headache, vomiting, myalgia, cough, splenomegaly, ↑GGT ↑ALT	**64/256** **16/128**	0/16 16/32	16/8 0/8	16/25 6 16/12 8	0/64 0/64	0/32 16/8	32/32 0/16	0/0 0/0	0/256 0/256	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
12	37/F	A. removed from ear	No	No	No	Chills, headache, vomiting, myalgia, cough, ↑GGT ↑ALT	**0/16** **128/32**	0/0 0/0	0/0 64/0	0/0 32/16	0/0 16/32	0/8 0/0	0/8 32/16	0/0 0/0	0/32 0/64	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
13	20/F	Tick bite	No	Yes	No	Headache, chills, myalgia	**32/16**[d] **64/16**	32/0[d] 32/0	32/0[d] 64/0	64/0[d] 128/0	32/8[d] 64/8	0/0 0/0	0/0 0/0	32/0[d] 64/0	0/0	512/0 512/0	0/0	0/0	0/0
14	55/F		No	No	No	Chills, headache, myalgia, diarrhea, thrombocytopenia	**16/32** **128/16**	16/16 16/16	16/16 16/16	16/16 16/16	16/16 64/0	16/16 16/16	16/0 64/0	32/0 64/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0	0/0 0/0
15	29/M		Yes[b]	No	No	Chills, headache, myalgia, cough, thrombocytopenia, ↑GGT ↑ALT	**16/16** **64/64**[d]	16/16 32/32[d]	32/16 32/32[d]	32/16 32/32[d]	16/0 64/64[d]	32/16 32/32[d]	16/0 64/64[d]	16/16 64/64[d]	0/0 0/0	256/0 256/0	256/0 256/0	0/0 0/0	0/0 0/0

Abbreviations: A., arthropod; Ig, immunoglobulin; R, rash; E, eschar; N, nodes; Rh, *Rickettsia helvetica*; Rc, *R. conorii* Indian; Rjap, *R. japonica*; Rhon, *R. honei*; Rslo, *R. slovaca*; AT1, *Rickettsia* strain AT1 from Japan; Rheil, "*R. heilongjiangii*"; Rfel, *R. felis*; G, strain Gilliam; Kw, strain Kawazaki; Kp, strain Karp; Ko, strain Kato.

[a]Immunofluorescence assay was completed by Western blot and cross-adsorption as described in the text. For typhus and spotted fever group antigens, the rickettsia considered potentially responsible for the infection are in bold type.

[b]Maculopapular over chest and back.

[c]Vesiculous on the legs.

[d]Antigens and sera used for Western blot assays.

[e]Antigens and sera used for Western blot and cross-adsorption assays.

previously been reported in Sangkhlaburi (24). *R. conorii* Indian is known as an agent of tick-borne rickettsioses prevalent in India, where it is associated with the dog tick (*Rhipicephalus sanguineus)* (25), which is found worldwide. However, an unknown *Rickettsia* sp. that is cross-reactive with *R. conorii* Indian and *R. helvetica* could also be responsible for the cases reported here. In particular, we have recently detected, by polymerase chain reaction, *Rickettsia* spp. from ticks that have bitten people in the Sangkhlaburi area, including *Dermacentor auratus* and *Dermacentor* sp. larvae (9). The pathogenic role of these rickettsiae has yet to be demonstrated.

Scrub typhus is essentially an occupational disease among rural residents in the Asia-Pacific region (2). This disease is often underdiagnosed or misdiagnosed when the classic eschar at the chigger bite sites and the rash are absent, as reported for two of our three patients (2). The severity of the disease varies from asymptomatic to fatal (up to 30%). Delayed or inappropriate treatment such as with third-generation cephalosporins, as reported for one of our patients, is associated with a severe outcome. The four major serotypes studied here have been shown to have sufficient cross-reactivity with antigens from other strains to be used for serologic diagnostic testing. In our patients, although the highest titers were obtained by using *O. tsutsugamushi* strain Gilliam antigens, other strains that share common epitopes and cross-react with this strain could be involved.

Patients with rickettsioses may have isolated fever or fever with nonspecific clinical and laboratory findings.

These diseases are easily misdiagnosed because rash or eschar (the hallmark for rickettsial diseases) is absent, the diseases are not recognized by local physicians, or the diseases have never been reported in the area. More studies are needed on tropical rickettsioses, in particular, molecular detection or rickettsial isolation from patient samples, complemented by detailed case reports. Studying possible vectors and animal reservoirs would provide estimates of the degree of zoonotic potential. Ultimately, such studies will provide the basis for determining prevalence of rickettsiosis in the tropics and their effects on public health.

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Dr. Parola obtained his M.D. and Ph.D. degrees at the Faculté de Médecine, Marseille, France. This work is part of his postdoctoral research project at the Harvard School of Public Health, Boston, Massachusetts, USA. His research interests include tropical diseases and medical entomology.

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Eliminating Trachoma in Areas with Limited Disease

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The common wisdom is that a trachoma program cannot eliminate ocular chlamydia from a community, just reduce infection to a level where blindness would be minimal. We describe the success of multiple mass antibiotic treatments, demonstrating that complete elimination of infection may be an attainable goal in an area with modest disease.

The World Health Organization (WHO) and a number of its partners have initiated a program to eliminate blinding trachoma by the year 2020 (1). Many healthcare workers feel that attempting to eradicate the ocular strains of chlamydia that cause trachoma (serovars A, Ba, B, and C) would be unrealistic and perhaps even unnecessary. A more attainable goal would be to reduce clinically active trachoma to some threshold, below which scarring and blindness would never occur or at least would become so rare that trachoma would no longer be a major public health concern (2).

Although in common usage the terms eradication and elimination can be synonymous, in the field of public health, they are not (3). Both terms imply reduction of incidence to zero. However, eradication applies to the whole world, whereas elimination applies to a defined geographic area and would require further monitoring; in a sense, elimination can be viewed as a local eradication (4). In practice, WHO has allowed an even looser usage of the term elimination: for example, leprosy elimination is defined as a prevalence of <1 case in 10,000 population, and tuberculosis elimination is an incidence of <1 case in 100,000 persons per year (5,6). WHO is currently in the process of defining such a level for trachoma.

Defining an appropriate target for trachoma elimination is particularly difficult because infection itself is rarely monitored. Control programs rely almost exclusively on the clinical examination because the most sensitive chlamydial tests are expensive and not widely available in trachoma-endemic areas. The clinical examination is certainly a reasonable tool to assess whether ocular chlamydia is hyperendemic in a community. However, the examination may not be an accurate indicator of infection when disease prevalence is low, as is often seen after treatment (7–11). The follicles so characteristic of clinically active trachoma may linger even when chlamydia is no longer detectable by using the most sensitive laboratory techniques (7,12). The few studies that have tracked ocular chlamydial infection using DNA amplification tests suggest that a single mass antibiotic distribution is very effective, much more successful than a clinical survey would indicate (9,10).

Could ocular chlamydia be eliminated with multiple treatments? A mathematical model has shown that periodic treatments could theoretically eliminate infection even without a perfect antibiotic or perfect coverage of the population (13). This same model predicts that annual treatment in areas with moderate amounts of trachoma should progressively reduce the prevalence of ocular chlamydia in a community. To date, however, no reports of the efficacy of multiple annual treatments on infection have been published.

The Study

We monitored trachoma prevalence in a village in Western Nepal for 3 years, using both a clinical grading system and nucleic acid amplification tests. Three annual azithromycin (20 mg/kg) treatments were distributed to all children ages 1–10 years in the village (Figure). All children were examined biannually, and the conjunctivae of a stratified random sample of children were swabbed and later tested for *Chlamydia trachomatis* DNA. At the final visit, 6 months after the last treatment, every child was examined, and their conjunctivae were swabbed. Before the first treatment, 39% had active infection determined by the clinical examination, and an estimated 26% (95% confidence interval [CI] 16% to 35%) were infected with chlamydia. At the final, May 2001 visit, 7 (4%) of 187 pediatric cases were clinically active. Only 1 child of the 187 (0.5%) had evidence of chlamydia by polymerase chain reaction.

Conclusions

This study suggests that local elimination of the ocular chlamydia that causes trachoma may be possible in a village with moderate baseline disease. After three annual treatments, only one infected child could be identified. Children are by far the most likely to harbor ocular chlamydia, and mathematical models imply that they will be the most difficult group to clear from infection (13,14). In fact, 1 year after mass azithromycin treatment in a vil-

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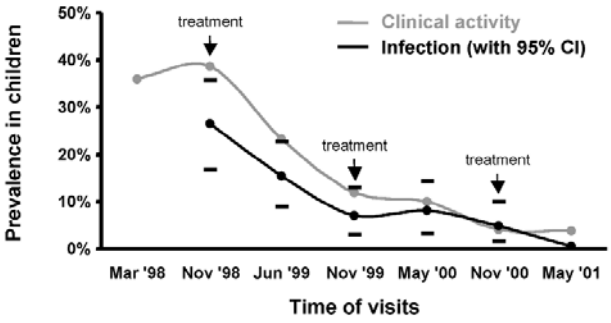

Figure. The prevalence of clinically active trachoma (gray curve) and ocular chlamydial infection, as determined by DNA amplification tests (black curve, with 95% confidence intervals due to stratified sampling) in children 1–10 years of age in a village in Western Nepal over time. All children were examined at each visit, so no sampling confidence interval is indicated. Likewise, conjunctivae of all children were swabbed for evidence of infection at the May 2001 visit.

lage in Egypt, more infection was identified in children 1–5 years old than in the rest of the community combined (9,15). Thus, the nearly complete absence of infection in children after three treatments implies that elimination is a possibility. Whether success in this village was due solely to our treatment program or due in part to a secular trend in the area, the results are encouraging.

Is elimination of ocular chlamydia necessary? It may not be for at least three reasons. First, repeat infections are almost certainly required to cause severe conjunctival scarring; occasional sporadic infections probably do not lead to blindness. Second, some investigators hope that if ocular chlamydia is reduced to a low enough level, the disease will have difficulty repopulating the community (population biologists call such a prevalence threshold an Allee effect [16]). While we see no reason for such a phenomenon a priori, if present, it would certainly establish a threshold target. Finally, bacterial, viral, and allergic conjunctivitides can occasionally mimic ocular chlamydia, so eradication of "clinically active" trachoma will never be possible.

Trachoma programs have already distributed more than 1 million doses of oral azithromycin, and some villages have received three annual treatments. How will we know when to stop? Now is the time to discuss the most appropriate target for trachoma programs and the most appropriate definition for trachoma elimination. The common wisdom is that complete local elimination of ocular chlamydia to zero in a defined geographic area is an unattainable goal, and that programs should settle for reducing the prevalence of ocular chlamydia to a level where little if any subsequent blindness would exist. These results from Nepal imply that the strict definition of elimination of ocular chlamydia in children may be an attainable goal, at least in areas with modest to moderate disease. Whether or not elimination is necessary is a separate question.

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Chronic Wasting Disease in Free-Ranging Wisconsin White-Tailed Deer

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Three White-tailed Deer shot within 5 km during the 2001 hunting season in Wisconsin tested positive for chronic wasting disease, a prion disease of cervids. Subsequent sampling within 18 km showed a 3% prevalence (n=476). This discovery represents an important range extension for chronic wasting disease into the eastern United States.

Chronic wasting disease (CWD) is degenerative and usually considered to be fatal in White-tailed Deer (*Odocoileus virginianus*), Mule Deer (*O. hemionus*), and Elk (*Cervus elaphus*) associated with the presence of transmissible protease-resistant prion proteins (PrP^{cwd}) (1,2). Although the transmission route of PrP^{cwd} is unknown, it may be transmitted in deer and elk by direct contact or indirectly from the environment (1,2). In experiments, clinical signs have appeared as early as 15 months after exposure (1) and include weight loss, anorexia, repetitive behaviors, hyperesthesia, and intractability. Signs progress to severe emaciation, extreme behavioral changes, excessive salivation, tremors, and mild ataxia (1,2). CWD was first recognized in captive Mule Deer in Colorado (3) and subsequently described in the free-ranging cervid populations of Colorado and Wyoming (1); prevalence in these disease-endemic areas varies spatially and among the three sympatric cervid species (4). Before its discovery in Wisconsin, CWD was detected in captive cervid farms in Colorado, Nebraska, South Dakota, Oklahoma, Kansas, Montana (USA), as well as Alberta, Saskatchewan (Canada), and South Korea (1). Apart from the contiguous areas of Colorado, Wyoming, and Nebraska, CWD had previously only been detected in two free-ranging Mule Deer from Saskatchewan, one Mule Deer from South Dakota, and in a number of Mule Deer from the western slopes region of Colorado (1). Previously, no cases of CWD were reported east of the Mississippi; however, subsequent to our research, CWD-positive cervids were found in Minnesota (captive Elk), Wisconsin (captive White-tailed Deer and Elk), and Illinois (free-ranging White-tailed Deer). Further, west of the Mississippi, the following CWD-positive animals have been found: Mule Deer in New Mexico and Utah; free-ranging Mule and White-tailed Deer in Saskatchewan, Canada; and captive Elk and White-tailed Deer in Alberta, Canada.

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The Study

In autumn of 1999 and 2000, the Wisconsin Department of Natural Resources (WDNR) submitted to the National Veterinary Services Laboratories (NVSL) (Ames, Iowa) brain material (obex) from 657 hunter-killed White-tailed Deer registered at hunter check stations across the state. None came from the study area we describe. Samples were tested for CWD prion by immunohistochemistry (IHC) (5). Prion was not detected in any samples. However, 3 of 445 White-tailed Deer shot in autumn of 2001 were positive for CWD. These deer were males, 2.5 years of age, and were shot within 5 km in south-central Wisconsin. WDNR subsequently conducted a sampling program to assess the distribution and prevalence of CWD in the vicinity of these three positive deer. We report the results of this sampling program.

Samples were collected from 500 adult (>1 year of age) White-tailed Deer within an approximate 18-km radius, and all samples were tested for CWD. Deer were submitted by hunters who were issued scientific collection permits, collected at roadside after vehicular collison, or collected by WDNR or U.S. Department of Agriculture sharpshooters. Data from collected deer included the geographic location based on the Wisconsin Public Land Survey System (township-range-section), sex, and age (estimated by using tooth eruption and tooth wear patterns [6]). Location of kill was indicated on a map by hunters during interviews by DNR staff. Samples of brain stem (obex) and retropharyngeal lymphatic tissue were fixed in 10% buffered formalin and submitted to NVSL for testing using IHC. We considered a deer to be CWD positive if either obex or retropharyngeal samples were IHC positive (1).

We used the spatial scan statistic provided by Kulldorff and Nagarwalla (7) (program SaTScan available from: URL: http://www3.cancer.gov/prevention/bb/satscan.html) to assess the presence and location of CWD clusters within the surveillance area. Location data were collected to the survey unit "section" (approximately 2.6 km^2). We pooled

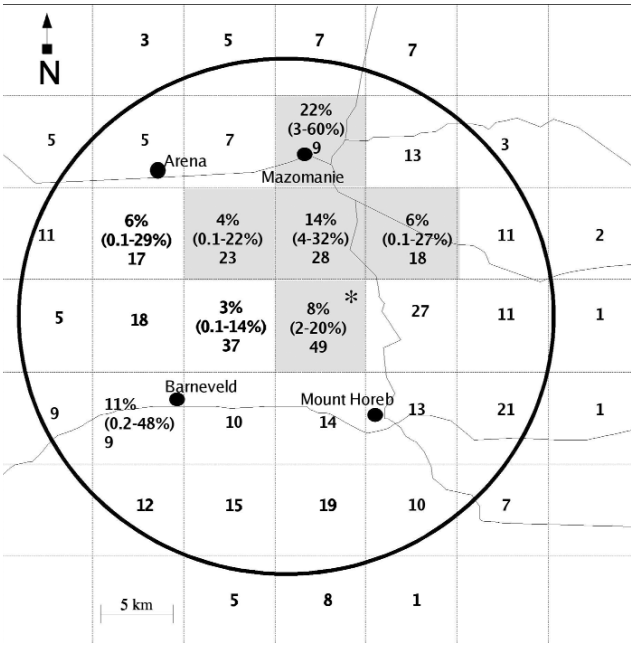

Figure. Spatial distribution of chronic wasting disease in White-tailed Deer sampled in Wisconsin (February–April 2002). Locations for sampled deer were recorded by using the Wisconsin Public Land Survey System (township-range-section); analysis was conducted on pooled 4X4 sections (41 km2), as indicated by the dashed grid lines. Prevalence, 95% confidence limits (CI), and sample size for each quadrat are indicated, as well as sample size only for quadrats in which positive deer were not detected. A cluster of higher than expected prevalence was detected in the north-central region of the sampling area indicated by shading (prevalence 9.4%, 95% CI 5.0% to 16.0%, n=127). The asterisk indicates the quadrat in which the three initial positive deer were found. The circle represents the targeted surveillance area.

locations into 4X4 section quadrats for analysis to compensate for sections from which no deer were collected. In a separate analysis, sex and age were assessed as predictors of CWD status by using logistic regression (function glm in program R v. 1.5.0; available from: URL: http://www.r-project.org) (8). Model selection uncertainty was incorporated into the odds ratio (OR) estimates by using model averaging (9).

Results and Discussion

From March 2 to April 9, 2002, samples were collected from 505 deer; however, 29 deer were not included in the analysis because of sample autolysis, inappropriate tissue submission, or lack of availability of appropriate tissues (e.g., deer with no intact cranium or those shot in the head). Of the remaining 476 deer (87 males, 386 females, and 3 for which sex was not recorded), 15 (3.2%; 95% confidence limit [CI] 1.7% to 5.1%) were IHC positive, 11 in both obex and retropharyngeal lymph node samples and 4 from lymph nodes only. We inferred that deer that were only lymph node positive were in the earlier states of infection (1). Estimated prevalence varied spatially within the surveillance area. A cluster of higher than expected prevalence was detected in the north-central region of the sampling area (prevalence 9.4%; 95% CI 5.0% to 16.0%; p=0.003; n=127) (Figure).

Prevalence did not vary by sex (males: 3.4%, 95% CI 0.1% to 9.7%, n=87; females: 3.1%, 95% CI 1.6% to 5.3%, n=386; male vs. female OR 1.1, 95% CI 0.56 to 2.19), a pattern consistent with Mule Deer sampled in Colorado and Wyoming (4). Increasing prevalence with age was suggested, although we could not distinguish whether the OR differed from 1 (OR 1.13, 95% CI 0.93 to 1.39). We had a small sample (n=32) of older animals (>5 years of age), which weakened our ability to detect an increase in prevalence with age statistically. Miller et al. (4) found that CWD prevalence increased with age in male Mule Deer and then abruptly declined in older age classes. We did not have a sufficient sample size to evaluate a sex difference in prevalence by age.

The known range of CWD was extended by its detection in Wisconsin, which is the first report of the disease east of the Mississippi River. Although we do not know how the free-ranging deer population of Wisconsin became affected by CWD, the most commonly suggested hypothesis is that CWD in Wisconsin may have emerged through importing of an affected cervid. The current enzootic of CWD in free-ranging deer and elk is paralleled by an enzootic in the captive cervid industry, and the relationship between CWD-affected elk farms and recent (2000–2002) diagnoses of CWD in free-ranging deer in Nebraska, South Dakota, and Saskatchewan remains under investigation (1). Elk were imported to Wisconsin from CWD-affected herds in Colorado during the 1990s, and recently (September and October 2002) captive White-tailed Deer were found to be positive on two separate farms in central and southern Wisconsin (10). Furthermore, during epidemiologic investigations of these positive farms, WDNR discovered that deer had escaped in March 2002 from one of these farms, one of which was later shot and found to be CWD positive (9). We stress that these positive captive deer are likely not the source of CWD in this free-ranging White-tailed Deer outbreak because of the captive deer's distance from the area where the CWD-positive free-ranging deer are (approximately 130 km). No direct evidence exists that CWD came to Wisconsin by the captive cervid industry. However, further investigation on possible links between CWD cases in captive and free-ranging cervids in Wisconsin is ongoing.

Conclusions

The state of Wisconsin is undertaking an integrated research, surveillance, and management program to determine the distribution of CWD in the Wisconsin free-rang-

ing deer population and eventually eliminating the disease from the known affected area of south-central Wisconsin (10,11). As of March 2003, a total of 39,636 deer had been sampled statewide for CWD as part of this surveillance and management program (data are available from: URL: http://www.dnr.state.wi.us/org/land/wildlife/whealth/issues/CWD/). Computer simulation of CWD dynamics in western cervid populations (12) indicated that CWD could severely reduce deer numbers. Disease transmission may occur at a greater rate and consequently have a larger impact on the population in the eastern United States, where White-tailed Deer densities are typically an order of magnitude larger than western deer and elk populations (e.g., deer densities in the CWD-affected area are estimated to be currently >20 deer per km^2) (WDNR, unpub. data). Deer and deer-related activities, such as hunting, wildlife viewing, and other social factors, are an important component of the Wisconsin culture and economy (approximately $1 billion/year) (13), prompting an aggressive research and management strategy to combat CWD in Wisconsin's free-ranging deer population.

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Human Metapneumovirus and Community-Acquired Respiratory Illness in Children

To the Editor: Stockton et al. have reported the detection of human metapneumovirus (HMPV) by using reverse transcriptase-polymerase chain reaction (PCR) in patients with influenzalike illness (1). These authors examined specimens submitted from patients, mainly adults, during winter 2000–01 and identified HMPV in 2.2% of patients with influenzalike illness who had tested negative for influenza virus and human respiratory syncytial virus (HRSV). Although several papers have been published on HMPV infection in children (2–4), the real impact of this virus on the health of the pediatric population remains to be determined. The data we obtained in the present study support the epidemiologic findings of J. Stockton et al. (1) and reinforce the notion that HMPV is a human pathogen associated with community-acquired acute respiratory tract infection (ARTI).

We investigated the occurrence of HMPV in children <3 years of age with ARTI during two consecutive winter seasons (November 2000–February 2001 and November 2001–February 2002) as part of a study to detect respiratory viruses (HRSV, influenza A and B viruses, parainfluenza virus types 1–4, and adenovirus) among the pediatric population. The study population comprised 565 children who were brought to Hospital Donostia, San Sebastián, Spain, with reported symptoms of ARTI, most of which (>80%) affected the lower respiratory tract. Of these children, 379 were hospitalized and 186 were discharged without admission. Hospital Donostia belongs to the public health system and is the main referral hospital for a population of 9,500 children <3 years of age. More than 97% of hospitalizations of children in our region occur in this hospital.

Nasopharyngeal aspirates were obtained and processed for cell culture by using rapid shell vial techniques on the MDCK, A-549, and LLC-MK2 cell lines. RNA was then extracted from the original samples by using phenol-chloroform (TRIzol LS Reagent, Invitrogen Corp., Carlsbad, U.K.) and was converted into cDNA with random primers by using M-MuLV reverse transcriptase (USB Corp., Cleveland, OH). Nested PCR was performed to detect HRSV, influenza, and parainfluenza viruses as previously described (5,6). The remaining cDNA was frozen at –80°C until subsequent use. We tested for HMPV in all samples that tested negative for the previously studied viruses, as well as in 100 randomly selected study samples that were positive for one or more of these viruses. HMPV detection was performed by PCR by using 5 μL of stored cDNA with primers derived from the F gene under previously described conditions (7). The PCR product (450 bp) from the HMPV-positive samples was sequenced in an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

In 411 (72.7%) of the 565 patients studied, at least one of the initially investigated viruses was detected. HRSV was found in 313 (55.4%) children, influenza in 44 (7.8%), parainfluenza in 36 (6.4%), and adenovirus in 32 (5.7%); 14 mixed infections were detected. Of 154 children with a negative result, HMPV detection was performed in 147 (95.5%), with a positive result in six children (4.1%). No HMPV was detected in any of the 100 samples previously positive for the initially studied respiratory viruses. Four of the six HMPV-positive children required hospitalization: a 7-month-old boy with pulmonary bronchodysplasia, rhinitis, and fever of 38.4°C (patient 1); a 20-month-old girl with previous obstructive pulmonary disease who had acute respiratory insufficiency along with generalized hypoventilation, crackles, wheezing, and radiologic images of air entrapment requiring bronchodilator administration (patient 2); a 16-month-old girl who had a febrile syndrome, basal crackles on pulmonary auscultation, and perihilar infiltrates (patient 3); and an 11-month-old boy with pneumonia of the upper left lobe (patient 4). The two remaining patients, a 7-month-old boy (patient 5) and a 9-month-old girl (patient 6), both with upper respiratory symptoms and clear chest, did not require hospitalization. In all six patients, outcome was favorable.

Analysis of the amplified sequences showed two clusters of HMPV. The first was composed of HMPV from patients 1, 3, 4, and 6 (GenBank accession nos. AY152846, AY152851, AY152850, and AY152847, respectively), and the second was composed of HMPV from patients 2 and 5 (GenBank accession nos. AY152849 and AY152848). The similarity among nucleotide sequences in the same cluster was ≥95% and oscillated from 86% to 88% when compared to those from a different cluster. During the second study season, we observed circulation of both clusters. When we compared these sequences of HMPV F gene obtained in Spain with those recently described in North America (7), we found that the sequences of the first cluster showed ≥95% similarity with the isolate CAN97-83 (GenBank accession no. AY145296), and the sequences from the second cluster showed ≥95% similarity with isolates CAN98-73 to CAN98-79 (GenBank accession nos. AY145287–AY145293), connecting the Canadian isolates to two well characterized groups of HMPV. Our results suggest that in Spain, as well as in other

places in the world (2,7), two major HMPV groups exist. The severity of the episodes observed varied from mild upper respiratory symptoms to severe infections requiring hospitalization for 2–6 days. Overall, as reported by other authors (2,8), the clinical picture provoked by HMPV was indistinguishable from that of other respiratory viruses. The fact that HMPV was not detected in any of the samples from patients also positive for other respiratory viruses suggests that coinfection is infrequent. The data reported in our study, obtained during two consecutive winter seasons in a pediatric population of southern Europe, allow us to estimate that the incidence of moderate or severe respiratory infections caused by HMPV is low and that the impact of the other respiratory viruses is considerably greater. Despite these results, we think that this new respiratory pathogen warrants surveillance. HMPV appears to be capable of provoking severe infections, and its role in human respiratory infections is still poorly understood.

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Puumala Virus Infection with Acute Disseminated Encephalomyelitis and Multiorgan Failure

To the Editor: Hantaviruses, which belong to the genus *Hantavirus*, family *Bunyaviridae*, are human pathogens that are prevalent worldwide (1). More than 16 different genotypes or serotypes have been identified (e.g., Puumala, Hantaan, Dobrava-Belgrade, Seoul, Sin Nombre). In western and central Europe, the predominant serotype is Puumala, which causes nephropathia epidemica. Puumala virus (PUUV) is spread by rodents and is transmitted to humans by inhalation or ingestion of food contaminated with rodent excreta (2). Nephropathia epidemica is endemic in western Russia, Finland, Sweden, France, Belgium, Germany, and former Yugoslavia. Reports of serologically verified nephropathia epidemica cases have also been published from Denmark, Norway, the Netherlands, and Austria (3). In Austria, the risk for infection seems to be restricted to special areas in Styria and Carinthia where *Clethrionomys glareolus*, the reservoir of PUUV in Austria, is endemic. The seroprevalence in Finland is 5% and 1.8% in Austria (4). The most common symptoms of nephropathia epidemica are fever, nausea, vomiting, headache, stomachache, back pain, tenderness in the kidney area, diarrhea or constipation, and red throat (5). PUUV infection may also lead to neurologic symptoms including meningoencephalitis, polyradiculitis, seizures, cerebral hemorrhage, urinary bladder paralysis, and hypopituitarism (6,7).

Our patient, a 43-year-old previously healthy man, had a temperature of 39°C and acute abdominal pain. Two days after the symptoms began, he was admitted to a regional hospital where acute renal failure and disseminated intravascular coagulation developed in the next 2 days. The patient was transferred to the Department of Medicine, Karl-Franzens University Graz, for intensive care. The patient worked in a factory, and he hunted in his spare time. A few days before his illness began, he had cleaned up his hut in the forest.

On admission to the intensive care unit, physical examination showed abdominal guarding and a body temperature of 39.2°C. Laboratory tests showed thrombocytes 36 G/L (140–440 G/L), creatinine 3.6 mg/dL (0.6–1.3 mg/dL), urea 132 mg/dL (10–45 mg/dL), D-dimere 1,558 µg/L (<200 µg/L), ATIII 67% (>75%), c-reactive protein (CRP) 237 mg/L (<9 mg/L), lactate dehydrogenase (LDH) 322 U/L, and slightly elevated liver enzymes. Computer tomography (CT) of the thorax showed bilateral opaci-

ties in the lungs and pleural effusion. In the CT of the abdomen, a thickened wall of the colon ascendens, an enlarged caecum, slightly enlarged kidneys, approximately 500 mL of ascites, and enhancement of the peritoneum were found. Gastroscopy and colonoscopy results were normal. In the ascites, protein of 3.1 g/dL and 1,000 cells/L with 73% neutrophils were detected. A few hours after admission to the intensive care unit, the patient's level of consciousness started to deteriorate, and respiratory failure and circulatory insufficiency with a blood pressure of 78/50 developed. He was intubated and ventilated, received catecholamines, and was empirically treated with meropenem and clarithromycin adjusted to renal function. Liquor examination showed elevated lactate (2.7 mmol/L; normal range 2.1 mmol/L) and elevated protein (67 mg/dL; normal range 45 mg/dL). Detailed cerebral spinal fluid testing did not show additional information. Despite antibiotic therapy, abdominal tenderness, organ functions, and laboratory test results worsened. Four days later antibiotic therapy was changed to ciprofloxacin and metronidazole adjusted to renal function and the patient was hemodialized. Because of increasing ascites, ileus, and raising CRP (from 216 to 391 mg/L) in the next 3 days, explorative laparotomy was performed, but no focus of infection could be found. One day after surgery, meningism and hyperreflexia developed. A brain CT showed wide areas of hypodensity bilateral in the white matter partially involving the cortex. Magnetic resonance imaging (MRI) showed bilateral areas of increased signal intensity located in the parietooccipital region extending to the frontal, temporal, and pons regions and associated with cerebral edema. The lesions predominately affected the white matter but, particularly in the occipital region, also involved the cortex. Because of the patient's history and his recent activities in his forest hut, serum samples were investigated for antibodies against PUUV, *Leptospira* sp., *Ehrlichia* sp., *Borrelia* sp., *Francisella tularensis*, *Bartonella henselae*, and *Coxiella burnetii*. PUUV antibodies were found to be positive (highest titers: immunoglobulin (Ig) M 1:64, IgG 1:8000) in an immunofluorescence test (Progen, Heidelberg, Germany) and an immunoassay (Mikrogen, Martiensried, Germany).

The patient further received catecholamines, hemodialysis, and mechanical ventilation. In the week after surgery, he improved clinically, and catecholamines, hemodialysis, and mechanical ventilation were stopped 15 days after initiation. One week later, a second brain MRI showed resolving abnormalities. Four weeks after admission to the intensive care unit, the patient left the hospital in good condition. Two months later, MRI of the brain was normal, and the patient was well at an 18-month follow-up.

A few reports of hantavirus infection with cerebral involvement have been published. Recently, a patient with acute disseminated encephalomyelitis following nephropathia epidemica was reported (2). Whereas this patient had acute renal failure and acute disseminated encephalomyelitis, our patient suffered from multiorgan failure with respiratory, circulatory, and renal insufficiency, paralytic ileus, disseminated intravascular coagulation, and acute disseminated encephalo-myelitis. In addition, in our patient, the disseminated encephalomyelitis involved parietooccipital, temporal, and frontal regions of the brain and also reached the brain stem. Other causes of acute disseminated encephalomyelitis such as multiple sclerosis, encephalitis caused by other infectious agents, uremic encephalitis, and hypertensive encephalitis could be ruled out.

In our patient, abdominal pain, ileus, thickened wall of the colon, and enlargement of the caecum mimicked acute abdomen, which has also been reported in two other cases of hantavirus infection (8). Usually hantaviruses are transmitted by inhalation of virus-containing particles originating from rodents urine, droppings, and saliva. Therefore, transmission can occur at any place that infected rodents have infested (9). In our patient, the probable source of infection was his housecleaning activities in his hut a few days before his illness. Since this hut served as a storage facility and was rarely entered, it was occupied by rodents.

In summary, PUUV infection should be considered in the differential diagnosis of multiorgan failure and acute disseminated encephalomyelitis, especially in patients from PUUV-endemic areas and typical history.

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Pregnancy and Asymptomatic Carriage of *Pneumocystis jiroveci*

To the Editor: Severe immunosuppression is the leading determinant host factor for *Pneumocystis* pneumonia (PCP) (1). However, PCP is not restricted to those who are severely immunocompromised. Molecular techniques based on the amplification of specific regions of *P. jiroveci* (human-derived *Pneumocystis*) DNA by using polymerase chain reaction (PCR) in noninvasive human samples suggest that the infection is common in other segments of the population that are immunocompetent or display a lesser degree of immune compromise (2,3). A mild or asymptomatic form of *P. jiroveci* infection, or a carrier state, likely develops in these persons, who may play a role in the circulation of *P. jiroveci* in the community while serving as silent reservoirs for transmission to susceptible persons. This description fits infants who acquire the primary *Pneumocystis* infection very early in life, patients with chronic respiratory disorders, elderly adults, and other groups (2,3). Extensive searches have been unsuccessful in detecting carriage of *P. jiroveci* DNA in noninvasive samples (i.e., nasal and throat swabs, saliva) from immunocompetent healthy adults (4).

Evidence suggests that latency of *P. jiroveci* is time-limited and that PCP is more likely an actively acquired infection (1). Characterization of potentially infectious reservoirs might lead to new intervention strategies to prevent transmission. Furthermore, the detection of *P. jiroveci* strains with mutations at the dihydropteroate synthase locus, which in other pathogens confer resistance to trimethoprim-sulfamethoxazole, suggests that resistance to this primary therapy of PCP may be emerging (1). New strategies for *P. jiroveci* prophylaxis may soon be needed.

Evidence suggests that normal pregnancy may be accompanied by changes in the immune response that may in part account for the successful growth and delivery of the "fetus hemi-allograft." A subtle shift from the response of Th1 (cellular immunity) CD4+ lymphocytes to a proportional increase in the Th2 (humoral immunity) CD4+ response can be detected (5). These responses have not been clearly explained but would most likely occur because of shifts in the production of cytokines, impairing defense against certain infections. Pregnancy's important hormonal changes (e.g., increases in the secretion of human chorionic gonadotropin, progesterone, estrogen, corticosteroids, α-fetoprotein, prolactin, and α-globulin) may also contribute to decreased resistance. While overt immune deficiency is difficult to detect, an increase in some viral infections has been documented, which may indicate a gentle form of depressed immune response (6). In addition, this physiologic compensation generates an increase in illness and death from other infections that require a protective Th1 response as, for example, tuberculosis, malaria, American trypanosomiasis, leishmaniasis, toxoplasmosis, lysteriosis, and pneumocystosis. Reports indicate that illness in HIV-infected women with PCP is greater when the women are pregnant (7). However, no data show that pregnant women may be asymptomatic carriers of *P. jiroveci*.

A prospective, pilot study of 33 third-trimester, pregnant, asymptomatic healthy women and 28 healthy women within 15 days of a menstrual period (controls) was conducted. Participants were followed at an outpatient clinic in Santiago during January through March 2002. Ages were 14–39 years (median 26 years) for pregnant women and 17–45 years (median 28 years) for controls. Previous pregnancies ranged from 0 (n=10) to 4 (median 1) for pregnant women and from 0 (n=9) to 3 (median 1) for controls. *P. jiroveci* was detected in deep nasal swab samples in a nested-PCR procedure by using oligonucleotide primers pAZ102E and pAZ102H. (These primers were designed for the gene encoding the mitochondrial large subunit rRNA of rat-derived *Pneumocystis* [*P. carinii*] that amplifies all forms of *Pneumocystis* and internal primers pAZ102X and pAZ102Y, specific for *P. jiroveci*.) DNA extraction was performed with a commercial kit (QIAamp DNA mini kit; Qiagen Inc., Valencia, CA). Positive, negative, and internal control primers, directed to the human globin gene to detect sample inhibition and verify successful extraction, were used during the DNA amplification procedure. Samples were processed under a laminar flow hood to prevent contamination, and PCR assays were repeated twice. The Ethics Committee of the University of Chile School of Medicine approved the study.

Five (15.5%) of the 33 pregnant women had *P. jiroveci* DNA in their nasal swab samples versus none (0%) of the 28 nonpregnant controls (p=0.04 by 1-sided Fisher exact test). Immunologic parameters were not tested. The *P. jiroveci*–positive women were all multiparous with 1 (n=2), 2 (n=2), or 3 (n=1) previous pregnancies.

These results suggest that pregnancy is a host factor that favors asymptomatic nasal carriage of *P. jirovec*. However, PCR detection of *P. jiroveci* DNA in the nares of pregnant women does not necessarily indicate either a mild active pulmonary infection or viable or transmissible organisms. In animal models, detection of *P. carinii* DNA in nasal and oral samples is a good indicator that *Pneumocystis* is in the lungs (8).

These results also support the hypothesis that pregnant women who nasally carry *P. jiroveci* may play a role as contagious sources for susceptible persons, especially their immunologically naive newborn infants. This hypothesis warrants further study. Mother-to-infant transmission may explain the accumulating evidence that the primary infection is widely acquired very early in life (9). Recent animal model studies have documented the early acquisition of *P. carinii* (within 1 to 2 h after birth) in neonatal rats, likely transmitted by the dams (10). Evidence of mother-offspring transmission would be clinically relevant for infants born to HIV-infected mothers, who currently rely on empiric anti-*Pneumocystis* chemotherapy started at 1 month of age as their only prophylactic option.

Acknowledgments

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First Evidence of *Aedes albopictus* (Skuse) in Southern Chiapas, Mexico

To the Editor: The mosquito *Aedes albopictus* (Skuse, 1894) was first identified in the Americas in Texas in 1985 (1,2). That year, this newly introduced species had dispersed widely in Texas and was implicated in the transmission of dengue virus (3). Later, the first states in Mexico that were infested by *Ae. albopictus* were along the northern Mexican border: Coahuila, Nuevo Leon, and Tamaulipas (4,5; J.P. Martínez-Muñoz, thesis). In 1997, this species was reported farther south in Veracruz (6). Although *Ae. albopictus* was expected to spread to southernmost Mexico, this mosquito has never been reported there until now. We have confirmed *Ae. albopictus* in the city limits of Tapachula, southern Chiapas, Mexico.

On September 13, 2002, one of the authors, who resides in Tapachula, was bitten by a mosquito. He collected the specimen, which was later identified as *Ae. albopictus* by the Centro de Investigación de Paludismo (CIP). Nearby larval habitats were then comprehensively searched to collect the immature stages of the species; the sampling area was located at 14° 55' 22.5" north and 92°15' 05.7" west at an altitude of 220 m along the periphery of Tapachula. We found the following containers with larval stages of mosquitos: five water containers, two discarded tires (con-

taining 300–3,000 mL of water), one thermal bottle (250 mL), one plastic bottle (50 mL), and one bucket (2,500 mL). Larvae were placed in plastic bags and transported to CIP laboratories, where they were allowed to emerge to adults during 17 days. The fourth instar larval and pupal exuvias were fixed and identified to species according to Darsie (7) and Superintendência de Campanhas de Saúde Pública (8). Twenty-five female and male *Ae. albopictus* from these collections are available from CIP laboratory upon request.

Additional field collections are being conducted to establish the distribution range of this species along the Chiapas coastal plain, to determinate the entomologic levels of infestation, and to determine its susceptibility to insecticides. Considering the epidemiologic relevance of this discovery, we have notified the proper health authorities to take necessary control measures to reduce the possibility of increased dengue transmission and to prevent other arboviruses, such as West Nile virus (9), from being spread by this new species in southern Mexico.

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Virus Isolation and "Acute" West Nile Virus Encephalitis (Response to Huang et al.)

To the Editor: We read with interest a recent article in your journal, First Isolation of West Nile virus from a Patient with Encephalitis in the United States (1); in the report, we were unable to ascertain indisputable evidence that this patient had indeed acquired acute West Nile virus (WNV) encephalitis. In animals (2,3) and humans (4), West Nile virus can persist in the host even after the host has recovered from an acute WNV infection, presumably more so in the immunocompromised persons. Therefore, in the case described by Huang et al. (1), proving that the patient did not have a history of WNV infection is important, particularly because this patient is from a geographic area where WNV is known to exist. The findings at autopsy of perivascular lymphocyte cuffing in mammillary bodies of the brain are not the classic findings reported during the West Nile encephalitis outbreak in New York City (5). The immunoglobulin (Ig) G antibody against WNV, if it had been present, would have been useful in that IgG antibody in the absence of IgM antibody is indicative of past rather than acute infection.

The WNV copy numbers in clinical samples and clinical indices (leukocyte count) suggest that the virus multiplies in the setting of leukopoenia or immune suppression and cannot be definitive proof that it was an acute infection, unless a negative preillness sample was available. The cause of the transient viremia, whether acutely acquired or from increased proliferation in a chronic infection, needs to be clarified further. In the future, antigen detection will guide patient management decisions; therefore, the possibility of a human chronic carrier state warrants study.

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"Acute" West Nile Virus Encephalitis (Response to Krishnamoorthy et al.)

To the Editor: In a letter to the editor, Krishnamoorthy et al. question the diagnosis of "acute West Nile encephalitis" in our case report. We did not use the word "acute" in the paper, but the patient did in fact have an acute illness. We believe that this case report, in which West Nile virus (WNV) was isolated in cell culture, represents the best evidence for a WNV infection in a human in the United States. The diagnosis of West Nile encephalitis was based on clinical analysis (1); not everyone with the diagnosis undergoes an autopsy. In many instances, patients do recover. In our case, the patient had the clinical features of encephalitis consisting of unremitting fever associated with a rapid course of progressive confusion and lethargy followed by coma. In addition, increased depression of respiratory drive existed, pointing to brain stem involvement. We agree that the inflammatory changes in the brain were limited as compared to such changes in other reported cases of WNV; however, this limitation was attributable to the fact that the patient was both immunocompromised and neutropenic at the time of acute infection. Therefore, the usual inflammatory response cannot be expected. Even though the changes were limited, they were consistent with the histologic findings in previously published reports (1,2).

The second point by Krishnamoorthy et al. represents their hypothesis about a human chronic carrier state for WNV. Although a chronic carrier state is possible, the viremic period associated with arboviral infections is typically short (3). While one cannot rule out persistent infection with WNV, until our report attempts to recover the virus by isolation in North America in humans have been uniformly unsuccessful. Also, previous reports of successful WNV isolations by Israeli investigators in immunocompetent hosts (4) have been from blood specimens before seroconversion. These considerations indicate that the virus is not routinely found in the blood in substantial amounts by the time clinical symptoms consistent with WNV infection occur. We do not know, nor have we speculated, about the timing of the infection as the patient had no recollection of a mosquito bite. Tests for both immunoglobulin (Ig) G and IgM antibodies to WNV were negative in our patient. Because the patient was immunocompromised, a humoral response was not expected; therefore, this information cannot be used as

evidence that the patient had an acute infection. However, observations that the patient had no manifestation of encephalitis during a previous episode of neutropenia and that she had an acute febrile illness associated with neurologic signs of encephalitis point to an acute infection. The figure, in which WNV copy numbers are correlated with leukocyte count, is not intended to pinpoint the time of infection. However, as stated in the paper, this figure did show that the virus was rapidly cleared after resolution of neutropenia.

A report by Camenga et al. (5) demonstrated that mice, infected with WNV develop only an inapparent infection. These mice will invariably die of fulminant encephalitis if only a single dose of cyclophosphamide is given. However, mice treated with one dose of cyclophosphamide demonstrate inflammatory changes in the brain. If a second dose of the drug is administered 5 days after infection, inflammation is completely suppressed in mice. Although mice are immunologically different from humans, this work, done almost 30 years ago, supports the argument for an acute infection in the current case report. If the patient in our study was a chronic carrier, she should have had manifestations of acute West Nile encephalitis immediately following the first course of combination chemotherapy, which was much more immunosuppressive than cyclophosphamide alone. This fact reemphasizes our major point in the article that patients who are immunocompromized and undergoing chemotherapy, which may cause neutropenia, should take extra precautions against being exposed to WNV.

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Instructions for Infectious Disease Authors

Letters

This section includes letters that present preliminary data or comment on published articles. Letters (500–1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

Correction Vol. 9, No. 4

In the article, "Antimicrobial Drug Prescriptions in Ambulatory Care Settings, United States, 1992–2000" by Linda F. McCaig et al. errors occurred on pages 432, 434, and 446. On page 432, the correct affiliations are as follows: Linda F. McCaig, National Center for Health Statistics, Centers for Disease Control and Prevention (CDC), Hyattsville, Maryland, USA; Richard E. Besser and James M. Hughes, National Center for Infectious Diseases, CDC, Atlanta, Georgia, USA. In the abstract, the change in antimicrobial prescribing rate for amoxicillin/clavulanate is +69%. On page 434, second paragraph, Results section, the correct first sentence appears below:

During the study period, the antimicrobial prescribing rate at all ambulatory care visits declined for amoxicillin and ampicillin (–43%; $p<0.001$), cephalosporins (–28%; $p<0.001$), and erythromycin (–76%; $p<0.001$) (Figure 5); the prescribing rate rose for azithromycin and clarithromycin (+388%; $p<0.001$), quinolones among persons ≥15 years (+78%; $p<0.001$), and amoxicillin/clavulanate (+69%; $p=0.004$) (Figure 6).

On page 436, the correct caption to Figure 6 appears below:

Trends in increasing annual antimicrobial prescribing rates by drug class—United States, 1992–2000. Note: trend for amoxicillin/clavulanate $p<0.001$; for quinolones among persons ≥15 years, $p<0.001$; for azithromycin and clarithromycin among all ages, $p<0.001$.

The corrected article appears online at http://www.cdc.gov/ncidod/EID/vol9no4/02-0268.htm.

We regret any confusion these errors may have caused.

Probiotics and Prebiotics: Where Are We Going?

Gerald W. Tannock, editor
Caister Academic Press,
Wymondham, U.K., 2002
ISBN: 0-9542464-1-1
Pages: 336
Price: $180

"Probiotics and prebiotics have become part of the lexicon of food technologists," writes Gerald W. Tannock, editor of the provocative new book, Probiotics and Prebiotics: Where Are We Going? Probiotics were defined by Fuller in 1989, as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance." The concept of probiotics is not new, however. Approximately 100 years ago, Elie Metchnikoff, the father of immunology, investigated intestinal microbes as causative agents in aging, a process he called "autointoxication." He believed that lactic acid–producing bacteria (such as those found in yogurt) would suppress the growth of more proteolytic, autointoxicating bacteria.

Prebiotics have been defined as "nondigestible food ingredients (usually carbohydrates) that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon." Potential prebiotics have included bifidobacteria, indigenous microbes epidemiologically associated with long life and other healthful conditions.

The concept of probiotics has been developing over recent decades, and the use of prebiotics extends this idea. Although the ideas are intriguing, the central theories to be tested and the tools necessary to test them have been lacking. This book contributes substantially to addressing these difficulties. The opening chapter, by Tannock, is rich in ideas and sets the appropriate tone for the rest of the book. The other nine chapters, by 20 other authors from seven countries, address both hypotheses and specifics in state-of-the-art reviews.

The central issue in this field is the following: how can the metabolic activities of the bacterial population in the colon be manipulated to promote health? Rigorous scientific exploration of this question has been limited by two factors: the colonic biota (flora) is vast and also largely undefined. In consequence, many studies, fueled by commercial self-interest, have lacked the stringency necessary for true scientific advancement. Accordingly, a substantial portion of this book discusses the methods, current or being developed, that will help address these deficiencies. Improved methods hold the promise of better defining which bacteria are present, distinguishing how much the biota varies from person to person, and measuring how well persons respond to probiotics and prebiotics.

Another important issue is exploring the relationship of the microbial biota and the host, especially the healthy host; such microecologic studies are critical to understanding potential microbial contributions to disease. Again, development of standard methods would permit these assessments; without extensive cataloging, we cannot establish the baseline.

A third and related issue is defining conditions that might be ameliorated by probiotic or prebiotic therapies. The authors provide a long list of such diseases, including colon cancer, inflammatory bowel disease, and some less obvious candidates such as osteoporosis and atopic diseases (for example, asthma). Some researchers have hypothesized that these latter diseases result from a childhood deprived of specific pathogens, the "hygiene hypothesis." Use of probiotics and prebiotics has been advanced as one solution to that problem.

In summary, Probiotics and Prebiotics is an important book, from which I have learned much. One deficiency, however, is the book's remarkable absence of discussion about the role of these agents in the selection of particular bacterial populations. The focus of the book, and of the field, is largely on metabolism, but any of the anticipated therapies, especially prebiotics, will select for particular bacterial species or phenotypes. A greater focus on the biology of selection in this milieu would have been helpful. Nevertheless, this limitation does not substantially reduce the great utility of this volume to those interested in ecology, microbiology, medicine, or nutrition. This book explores a field that is out of the mainstream of human biology and medicine but deserves to be more central. For a field often marked by hyperbole because of commercially based conflicts of interest, this book is appropriately subdued and scientifically balanced. The editor and authors should be credited for their scholarly approach.

Martin J. Blaser*
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Instructions for Infectious Disease Authors

Book Reviews

Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

Eighth International Course on Dengue and Dengue Hemorrhagic Fever: Still a Menace to the Public Health of the Americas

The Pan American Health Organization (PAHO)/World Health Organization (WHO) Center for Viral Diseases and PAHO/WHO Center for Medical Malacology and Vector Control of the "Pedro Kourí" Tropical Medicine Institute (IPK) in Havana, Cuba, the Ministry of Health, PAHO, and the Special Programme for Research and Training in Tropical Diseases (TDR)/WHO announce the Eighth International Course on Dengue and Dengue Hemorrhagic Fever: Still a Menace to the Public Health of the Americas. On the 170th anniversary of the birth of Carlos J. Finlay, the Cuban scientist who discovered the transmitting agent of yellow fever, the *Aedes aegypti* mosquito, vector control continues to be the only alternative available to stop the spread of the dengue. The 8th biannual International Course on Dengue will be held in Havana, Cuba on August 11 to 22, 2003.

The objective of the course is to provide a forum for participants and presentations by lecturers who specialize in virology, epidemiology, vector control, immunology, sociology, and medical care and have experience with dengue fever and dengue hemorrhagic fever (DHF). Participants will be able to review up-to-date facts about dengue.

All presentations will be in Spanish. Practical sessions will be divided into three groups (laboratory diagnosis; entomology, vector control, and community participation; and clinical and pathologic aspects) according to the professional profile of attendants. Topics will include dengue and DHF in the Americas, new viruses in old areas and old viruses in new areas, West Nile virus in the Americas, epidemiology of dengue, clinical and therapeutic aspects of dengue and DHF, healthcare organization in emergency situations, laboratory diagnosis, molecular biology of dengue viruses, molecular evolution/epidemiology of dengue, bioinformatics and dengue, immunopathogeny and physiopathology, T-cell responses, vaccine development update, and social sciences, vector control, and dengue prevention.

Applications should be sent before July 1, 2003, to: Prof. Maria G. Guzman, Instituto Pedro Kouri, Autopista Novia del Mediodia, Km. 6 P.O. Box Mnao 13, Ciudad Habana, Cuba; fax: 53-7-2020460; telephone: 53-7-246051 or 53-7-2020633; email: lupe@ipk.sld.cu. Applications should include the name and address of applicant, telephone and fax numbers, and email; a summarized curriculum vita; and the practical session of preference. Registration is $1,000 (U.S), which includes registration, course materials, welcome cocktail, and farewell dinner.

For additional information visit the Web site: http://www.ipk.sld.cu/eventosipk/curso-dengue1.htm (English) or http://www.ipk.sld.cu/eventosipk/curso-dengue1.htm (Spanish).

2003 Annual Conference on Antimicrobial Resistance: Science – Prevention – Control

Hyatt Regency Bethesda
Bethesda, Maryland
June 23–25, 2003

The 2003 Conference on Antimicrobial Resistance will be held June 23–25, 2003, in Bethesda, Maryland. In addition to exploring the science, prevention, and control of antimicrobial resistance, participants will define issues and potential solutions to this problem.

The conference is sponsored by the National Foundation for Infectious Diseases (NFID) in collaboration with nine agencies, institutes, and organizations involved in conducting or promoting research, prevention, and control of antimicrobial resistance.

The deadline for online submission of abstracts for oral and poster presentations is April 1, 2003. Registration fee is $350 until May 16. After that date, the fee will be $400.

For additional information, contact NFID, 4733 Bethesda Avenue, Suite 750, Bethesda, MD 20814-5278; telephone: 301-656-0003, extension 12; fax: 301-907-0878; email: resistance@nfid.org. Program announcements and forms for abstract submission, registration, and hotel reservations are also available at http://www.nfid.org/conferences/resistance03/

Upcoming Infectious Disease Conferences

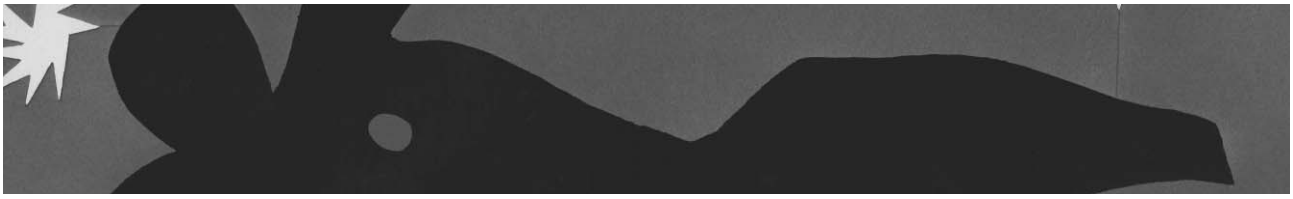
May 1–3, 2003
ISAAR 2003—4th International Symposium on Antimicrobial Agents and Resistance
Antimicrobial Treatment in the 21st Century: Current Challenges and Future Strategies
Seoul, Korea
Contact: Ms. Susan Chung
Phone: 82-2-3410-0327
Fax: 82-2-3410-0023
Email: susan@ansorp.org
Website: http://www.ansorp.org/isaar2003/ intro.htm

May 7–11, 2003
8th Conference of the International Society of Travel Medicine
New York City
Contact: Lisa Astorga, Conference Manager

May 10–13, 2003
13th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)
Glasgow UK
Contact: Administrative Secretariat
+41 61 686 77 11
Email: info@akm.ch
Website: www.escmid.org/eccmid20

May 27–30, 2003
Global Health Council's 30th Annual Conference
Our Future on Common Ground: Health and the Environment
Washington, DC
Website: http://www.globalhealth.org

May 29–30, 2003
ISC Disease Management Series International Conference
Surgical Infections: Prevention and Management
Moscow, Russia
Contact: Dr. Dr. Dmitry Galkin
PO Box 60, Smolensk, 214019, Russia
Tel.: 7 0812 611301/611327
Fax: 7 0812 611294
E-mail: galkin@antibiotic.ru
Website: http://www.antibiotic.ru



Henri Matisse (1869-1954). Icarus (from the illustrated book, Jazz, published in 1947 by E. Tériade). Copyright 2003 Succession H. Matisse, Paris / Artists Right Society (ARS), New York

"…send me a white cane," Henri Matisse exhorted his assistants when he completed the compositions for his illustrated book Jazz. The artist was nearly blinded by working with intense color under the brilliant Mediterranean light of the south of France (1). To protect against glare, he used overstated hues and intense blacks. To overcome incapacitating illness, he invented a new medium, "drawing with scissors." Cutting shapes from prepainted paper, he formed the contour and the internal area of a shape simultaneously, eliminating as he put it, "the eternal conflict between drawing and color" (2). These cutouts, begun as compensation for illness (duodenal cancer) that confined him to a wheelchair, became another creative peak near the end of the artist's life.

Matisse started to paint while convalescing from appendicitis at age 20. He became so captivated by the joy of creative expression that within a year he abandoned his law aspirations and went to Paris to study art, in a period still reverberating with the color innovations of van Gogh, Gauguin, and Cézanne. Trained in the academic tradition by symbolist painter Gustave Moreau, Matisse used his love of the human figure and his solid footing in art history as a springboard to greatness. He became a leader of the Fauve movement, known for its radical, even violent, use of color. He broadened his artistic scope through study of Japanese prints, Persian ceramics, and Arabic designs and sought inspiration in Spain and Morocco (3). His long career as painter and sculptor was filled with restless experimentation, and in addition to innovative paper cutouts, his artistic efforts extended to tapestry, ceramics, stained glass, and murals. Along with Pablo Picasso, he became a pillar of 20th century art (4).

In Jazz, Matisse's cutout forms are mingled with meditations on random topics, elaborately scrolled and interspersed throughout the composition. In this syncopated design (perhaps the visual counterpart of jazz music, which the artist defined as "rhythm and meaning"), figures are chromatic and rhythmic improvisations distilled to pure form (1). Spare and geometric, they are filled with undulating movement and circular rhythm. Even though their range is deliberately reduced, the colors are exuberant and provocative, and the harmonious compositions are filled with almost palpable light (2).

In "Notes of a Painter," Matisse reflected that his goal as an artist was to uncover and record with balance and purity the "essential character" of things beneath their external appearance. Icarus, on this cover of Emerging Infectious Diseases, is one of the most famous figures in Jazz. The cutout interprets the symbolic journey of Daedalus' son (5) and depicts the fall of the mythologic adventurer from the azurean skies amidst "either stars or bursts of artillery fire" (perhaps reflecting the artist's consternation in the aftermath of World War II). The pure form of the cutout, and the color that constitutes rather than clothes the form, captures the essence of human exploration.

Icarus' stretched-out arms negotiating flight, the fiery heart cloaked in the vibrant black of its aspirations, the bright chunks of sun that proved the man's demise freeze in a moment of exhilaration. About to end, the euphoric moment turns somber. The head is tilted away from the sun's splendor toward the pedestrian view below. The gliding figure, closing its celestial dance and filled with exalted vertigo, is laden with the certainty of the fall.

Our age has transformed Icarian and heliotropic quests into space exploration. We orbit the globe, defying the sun and the forces of gravity, for we still long for the charged moment of discovery that comes from roaming the earth and beyond. Yet, we have conquered neither gravity nor the mundane hazards at our destinations. Like Daedalus' crude fabrications, our wings still melt in the heat, and during travel, we fall prey to biologic hazards, exotic microbes. Be it emergent viruses (such as the cause of severe acute respiratory syndrome) or common intestinal bacteria (including Aeromonas spp.), the most insistent plague of travelers, these hazards slow the journey and limit the height of human exploration.

Polyxeni Potter

1. Matisse H. Jazz. New York: George Braziller, Inc.; 1992.
2. Elderfield J (Dir). Henri Matisse. Masterworks from the Museum of Modern Art. New York: The Museum of Modern Art; 1997.
3. Zuffi S. One thousand years of painting. Spain: Borders Press; 2001.
4. Janson HW, Janson AF. History of art. New York: Harry N. Abrams, Inc.; 2001.
5. Kaniamos P. The tale of Daedalus and Icarus as described in Greek mythology [cited 2003 March 24]. Available from: URL: http://www.skyfly.cz/zajimavo/icarus.htm.

EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.9, No.6, June, 2003

Upcoming Issue

For a complete list of articles included in the June issue, and for articles published online ahead of print publication, see http://www.cdc.gov/ncidod/eid/upcoming.htm

Look in the June issue for the following topics:

Histopathologic Features of *Mycobacterium ulcerans* Infection

Serogroup W-135 Meningococcal Disease during the Hajj, 2000

Parachlamydiaceae as Rare Agents of Pneumonia

Gnathostomiasis: An Emerging Imported Disease

Imported West Nile Virus Infection in Europe

Hantaviruses in the Czech Republic

Corynebacterium ulcerans Diphtheria in Japan

Rift Valley Fever Virus Infection among French Troops in Chad

Poor Potential Coverage for 7-Valent Pneumococcal Conjugate Vaccine

Tick-Borne Encephalitis with Hemorrhagic Syndrome, Novosibirsk Region,

Anthroponotic Cutaneous Leishmaniasis, Kabul, Afghanistan

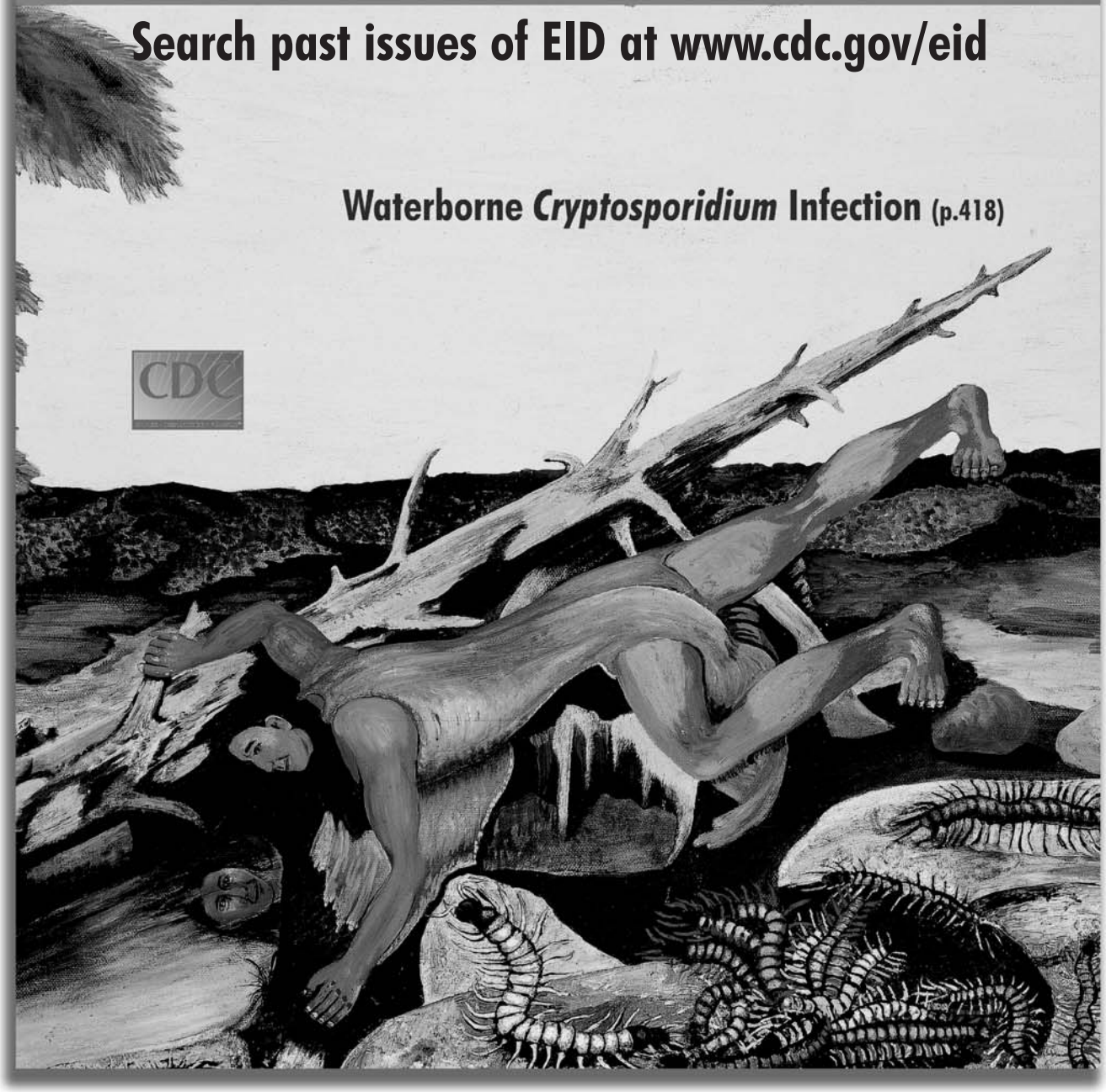
Salmonella in Birds Migrating through Sweden

EMERGING INFECTIOUS DISEASES

EID Online www.cdc.gov/eid

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.9, No.4, April 2003

Search past issues of EID at www.cdc.gov/eid



EMERGING INFECTIOUS DISEASES www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and *West Nile virus* infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

Editorial Policy and Call for Articles

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal has an international scope and is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the Editor at 404-371-5329 (tel), 404-371-5449 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features the following types of articles: Perspectives, Synopses, Research Studies, Policy and Historical Reviews, Dispatches, Commentaries, Another Dimension, Letters, Book Reviews, and News and Notes. The purpose and requirements of each type of article are described in detail below. To expedite publication of information, we post journal articles on the Internet as soon as they are cleared and edited.

Chinese, French, and Spanish translations of some articles can be accessed through the journal's home page at http://www.cdc.gov/eid.

Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. Begin each of the following sections on a new page and in this order: title page, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and e-mail address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Printed manuscript should be single-sided, beginning with the title page. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables and Figures. Create tables within MS Word's table tool. Do not format tables as columns or tabs. Send graphics in native, high-resolution (200 dpi minimum) .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) format. Graphics should be in a separate electronic file from the text file. For graphic files, use Arial font. Convert Macintosh files into the suggested PC format. Figures, symbols, letters, and numbers should be large enough to remain legible when reduced. Place figure keys within the figure. For more information see EID Style Guide (http://www.cdc.gov/ncidod/EID/style_guide.htm).

Manuscript Submission. Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch) and verifying that the final manuscript has been seen and approved by all authors. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases website (www.cdc.gov/eid).

Manuscript Types

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch of first author—both authors if only two. Report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words) and a brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be 1,000–1,500 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed two); and a brief biographical sketch of first author—both authors if only two. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but should not include figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. This section includes letters that present preliminary data or comment on published articles. Letters (500–1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

Book Reviews. Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted on the journal Web page only, depending on the event date.)

Conference Summaries. (500–1,000 words) of emerging infectious disease conferences may provide references to a full report of conference activities and should focus on the meeting's content rather than on individual conference participants.